FROM FIELD TO RUMEN: FOLIAR FUNGICIDE APPLICATION ON CORN AND ITS EFFECTS ON THE CORN PLANT, CORN SILAGE, AND HOLSTEIN COW PERFORMANCE

BY

CAROLINE C. KALEBICH

THESIS

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Master's Committee:

Assistant Professor Felipe C. Cardoso, Chair
Professor James K. Drackley
Professor Emeritus Michael Murphy
Assistant Professor Daniel Shike

ABSTRACT

An increasing global population, decreasing amount of arable land available for crop production in the United States, and an increased global demand for protein in the human diet encourage crop and livestock producers to seek solutions to improve the efficiency of producing large crop yields. The interaction of fungi and corn plants in the field threaten yields, decreasing the efficiency of food production and the nutritive quality of feedstuffs for ruminants. Fungicides can assist corn plants in protection from fungal infection by limiting yield losses and increasing the nutritive quality of the plant material. However, little is known about how various applications of fungicide on corn change the nutrients of individual parts of the corn plant, alter the fermentation of corn silage once ensiled, and affect the milk production when fed to dairy cattle. Therefore, the objectives of the present study were to investigate various applications of fungicide on: corn associated with the nutritive profile and growth of corn leaves, ears, stalks, and flag leaves; once ensiled, on the nutritive and fermentative profile of corn silage; and when corn silage is fed to dairy cattle on milk production, milk components, blood metabolites, and cow health. Corn from two growing seasons, 2014 and 2015, with different fungicide treatments was included in this study. Corn grown during the summer of 2014 was ensiled and fed to dairy cows, while corn grown during the summer of 2015 evaluated the plant and corn silage.

In 2014, treatments were as follows: corn silage with no application of foliar fungicide **(CON)**; corn silage received one application of pyraclostrobin and fluxapyroxad (PYR+FLUX) foliar fungicide (Priaxor®; BASF Corp.) at corn stage V5 **(V5)**; corn silage received one application of PYR+FLUX at corn stage V5 plus another application of PYR+FLUX at corn stage V8 **(V5/V8)**; corn silage received one application of PYR+FLUX at corn stage V5, one application of PYR+FLUX at corn stage V8, plus a third application of pyraclostrobin and

metconazole (PYR+MET) foliar fungicide (Headline AMP®; BASF Corp) at corn stage R1 **(V5/V8/R1)**. Corn was harvested at 31.2% DM and ensiled for more than 200 d before feeding. Treatments were fed to cows for 5 wk with only the last week being used for statistical inferences. Three contrast statements were used: contrast 1: CON vs. TRT compared control to the average of treatments fed corn silage sprayed with foliar fungicide (V5, V5/V8, and V5/V8/R1); contrast 2: V5 vs. V5/V8 compared the treatment fed corn silage sprayed at V5 to the treatment fed corn silage sprayed at V5 and V8; and contrast 3: V5/V8 vs. V5/V8/R1 compares the treatment fed corn silage sprayed at V5 and V8 to the treatment fed corn silage sprayed at V5, V8, and R1. No differences in DMI (19.5, 19.5, 20.8, and 20.4 kg for CON, V5, V5/V8, and V5/V8/R1, respectively) or milk yield (30.5, 31.2, 29.1, and 29.3 kg/d) were observed. However, cows in V5 when compared with cows in V5/V8 tended, to produce more 3.5% fat corrected milk (FCM; 32.42 and 28.58 kg/d, respectively) and energy corrected milk (ECM; 31.35 and 27.76 kg/d, respectively). Concentration of milk lactose tended to be greater for cows fed corn silage treated with foliar fungicide when compared with CON.

In 2015, the study was split into two parts, but the fungicide treatment was the same for both part one and part two. Treatments were as follows: control (**CON**), corn receiving no foliar fungicide application; treatment 1 (**V5**), where corn received a mixture of pyraclotrobin and fluxapyroxad foliar fungicide (Priaxor, BASF Corp.) corn vegetative stage 5 (V5); treatment 2 (**V5+R1**), where corn received two applications of foliar fungicide, a mixture of pyraclotrobin and fluxapyroxad at V5 and a mixture of pyraclostrobin + metconazole foliar fungicide (Headline AMP; BASF Corp.) at corn reproductive stage 1 (R1), and treatment 3 (**R1**), in which corn received one applications of pyraclostrobin + metconazole foliar fungicide at R1. Evaluators at R1 and R3 reported signs of Gray Leaf Spot and Northern Leaf Blight on the foliage. In part one, 24 individual corn plants from each treatment were collected R1 and R3 for weight and length measurement. At each collection, treatment corn plants were disassembled into leaves, stalks, flag leaf, and ears for nutrient analysis. The effect of foliar fungicide treatment, corn growth stage, and treatment by growth stage was evaluated on a dry matter basis. Interactions of fungicide applications on corn by collection time point were observed for the number of yellow leaves (0, 0, 0, and 0 at R1 and 0.85, 0.77, 0.42, and 0.44 for CON, V5, V5+R1, and R1 at R3 respectively; $P = 0.03$) and the height of the stalk (2.89, 2.94, 2.92, and 2.96 m at R1, and 2.50, 2.91, 3.05, and 2.80 for CON, V5, V5+R1, and R1 at R3; $P = 0.02$), with greater values for corn treated with fungicide than untreated. Corn stalks from corn treated with fungicide had greater concentrations of lignin compared with untreated (46, 56, 64, and 50 g/kg DM for CON, V5, V5+R1, and R1, respectively), with the greatest value from corn in V5+R1. Corn leaves from corn treated with fungicide had lower concentrations of ADF (333, 331, 283, and 330 g/kg DM for CON, V5, V5+R1, and R1, respectively) and NDF (569, 584, 524, and 554 g/kg DM for CON, V5, V5+R1, and R1, respectively) compared with untreated, with the lowest concentrations of ADF and NDF from corn in V5+R1. Interactions of applications of fungicide on corn by collection time point in corn leaves were observed for ADF (329, 335, 338, and 336 g/kg at R1, and 337, 326, 228, and 304 g/kg at R3 for CON, V5, V5+R1, and R1, respectively; $P = 0.008$), Na (14, 12, 10, and 7 g/kg at R1, and 6, 5, 7, and 5 g/kg at R3 for CON, V5, V5+R1, and R1, respectively; $P = 0.02$), and Cu (12, 12, 11, and 13 PPM at R1, and 15, 15, 18, and 17 PPM at R3 for CON, V5, V5+R1, and R1, respectively; $P = 0.03$).

In part two, the effect of treatment, ensiling time, and treatment by ensiling time was evaluated on a laboratory scale. Samples of the chopped corn were collected at harvest, prepared as 0.9-kg silos, and vacuumed sealed (28 × 36 cm). Chopped corn ensiled for 0 d was frozen on the day of harvest, while silos for 30, 90, and 150 d were left in the vacuum-sealed bags for each respective time frame and frozen for later analysis. Applications of foliar fungicide on corn ensiled as corn silage decreased dry matter (335, 319, 315, and 317 g/kg DM for CON, V5, V5+R1, and R1, respectively), but increased crude protein (81, 85, 82, and 87 g/kg DM for CON, V5, V5+R1, and R1, respectively), water soluble carbohydrates (38, 40, 46, and 52 g/kg DM for CON, V5, V5+R1, and R1, respectively), and lactic acid (46.5, 50.1, 50.9, and 55.0 g/kg for CON, V5, V5+R1, and R1, respectively). Applications of fungicide at R1 had the lowest lignin compared to treatments (20 g/kg DM for R1 vs 24, 24, 26 g/kg DM for CON, V5, and V5+R1, respectively), and corn silage in V5 had greater milk kg/MT DM (1631 kg/ton DM for V5 vs. 1511, 1585, and 1576 kg/MT DM for CON, V5+R1, and R1, respectively; $P = 0.04$). Length of ensiling postharvest affected the dry matter (327, 314, 325, and 320 g/kg for 0, 30, 90, and 150 d, respectively; $P = 0.03$), crude protein (81, 85, 84, and 86 g/kg for 0, 30, 90, and 150 d; $P < 0.0001$), and pH (5.74, 3.75, 3.80, and 3.80 for 0, 30, 90, and 150 d; $P < 0.0001$) of corn silage. Significant interactions between foliar fungicide applications on corn ensiled as corn silage and length of ensiling postharvest were observed for water soluble carbohydrates (80, 91, 111, and 125 g/kg at 0 d; 16, 17, 19, 22 g/kg at 30 d; 25, 25, 26, and 32 g/kg at 90 d; 31, 28, 27 and 32 g/kg at 150 d for CON, V5+R1, and R1, respectively; $P = 0.03$), and lactic acid (1.0, 0.5, 0.4, and 0.5 g/kg at 0 d; 54.3, 67.8, 64.4, 71.9 g/kg at 30 d; 63.4, 68.5, 69.2, and 71.1 g/kg at 90 d; and 62.7, 63.7, 69.7, and 76.6 g/kg at 150 d for CON, V5+R1, and R1, respectively; $P = 0.03$).

In conclusion, fungicide application on corn affected the nutritional profile differently depending on the part of the plant. Once ensiled, fungicide application on corn impacted the nutritional composition and fermentation of corn silage ensiled for varying lengths of time postharvest. Finally, corn silage from corn receiving foliar fungicide fed to cows impacted milk

production and composition. Cows fed corn silage receiving foliar fungicide treatment at V5 had greater FCM and ECM than cows fed corn silage receiving foliar fungicide treatment at V5 and V8. Results from 2015 corn indicate applications of fungicide on corn reduced the number of yellow leaves and increased the height of the corn stalk. Applications of fungicide on corn atV5 and R1 resulted in the greatest concentration of lignin in the stalk, but applications of fungicide on corn at both V5 and R1 reduced the concentration of ADF and NDF in the corn leaves. Applications of fungicide on corn ensiled as corn silage reduced the DM content, and applications of fungicide at R1 resulted in the lowest concentration of lignin in corn silage. Foliar fungicide treated corn and, then corn silage, increased the nutritive quality of the plant material and corn silage by decreasing the fibrous content, and resulted in increased FCM and ECM when fed to dairy cows.

Key words: Corn, Dairy Nutrition, Digestibility, Energy Corrected Milk, Fungicide, Fat Corrected Milk

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“Without a coach, people will never reach their maximum capabilities.”
Bob Nardelli, CEO of Home Depot

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List of Abbreviations

ADF	Acid detergent fiber
AOAC	Association of Analytical Communities
BCS	Body condition score
BUN	Blood urea nitrogen
BW	Body weight
CON	Control
Cu	Copper
DAMPs	Damage associated molecular patterns
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
DMIh	Demethylation inhibitors
DON	Deoxynivalenol
ECM	Energy corrected milk
ECM/DMI	Energy corrected milk / Dry matter intake (Feed conversion)
FCM	Fat corrected milk
FCM/DMI	Fat corrected milk / Dry matter intake (Feed conversion)
Fe	Iron
FLUX	Fluxapyroxad
FS	Fecal score
FUM	Fumonisin
GS	General appearance score
K	Potassium
kg	Kilogram(s)
LAB	Lactic acid bacteria
LS	Lameness Score
LSM	Least squares means
m	Meter(s)
MET	Metaconzole

Mg	Magnesium
MILK kg/ DM	Milk yield / Dry matter intake (Feed conversion)
min	Minute(s)
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber digestibility
NEFA	Non-esterified fatty acid
Ne_g	Net energy of growth
Ne_l	Net energy of lactation
Ne_m	Net energy of maintenance
NFC	Non-fibrous carbohydrates
NRC	National Research Council
OTA	Ochratoxin A
PAMPs	Pathogen associated molecular patterns
PTI	Plant triggered immunity
PYR	Pyraclostrobin
QoI	Quinol oxidation inhibitors
R1	Corn reproductive stage 1
R3	Corn reproductive stage 3
R4	Corn reproductive stage 4
S	Sulfur
SCC	Somatic cell count
SDHI	Succinate dehydrogenase inhibitors
SEM	Standard error of the mean
T-2	Trichothecene
TDN	Total digestible nutrient
THI	Temperature-humidity index
TMR	Total mixed ration

TRT	Treatment
V5	Corn vegetative stage 5
V8	Corn vegetative stage 8
VT	Corn vegetative stage tassel
Wk	Week(s)
WSC	Water soluble carbohydrates
Zea	Zearalenone
Zn	Zinc

CHAPTER I

LITERATURE REVIEW

World Perspective

World population steadily increased in recent years: in 2013 human population totaled 7.1 billion people; in 2014, 7.2 billion people; and in 2015, 7.3 billion people (FAO, 2015a). By the year 2050, the world's population is projected to reach 9.7 billion people (United Nations, 2015). In order to supply the 2050 projected population, future farmers will have to produce more food with fewer resources. Increases in the global efficiency of converting cropland to livestock products (milk, meat, and eggs) could help lessen the caloric dependency on cereal grain which is required to nourish the population (Gilland, 2002).

The Food and Agricultural Organization (FAO) estimated in 2014 total harvested land was greatest in China (36 million ha), followed by the United States (34 million ha), and Brazil (15 million ha) (FAO, 2015d). Of the cereal grains produced globally, maize is one of the most widely cultivated cereal grains important for both human and animal nutrition. On a global scale, the United States produced the most maize (327 million metric tons) in 2014, followed by China (195 million metric tons) and Brazil (14 million metric tons) (FAO, 2015d). Large yields of corn may be credited to plant hybrids, control of disease and insects, crop rotation, weather patterns, and soil quality. As the population grows, one time farmland will be converted to housing communities, further emphasizing the importance of crop production efficiency. From 2006 until 2010, 17% of the land in United States was arable for crop development, but declined recently to 16.6% (World Bank, 2016). With less land devoted to growing crops and a greater proportion of grain to be used for livestock feed to supply the increased demand of animal protein (Gilland, 2002), improved efficiency of crop production is crucial to limit devastating epidemics and crop yield losses (Knogge, 1996).

Fungus - a threat for corn

Yield losses due to fungal infections

In 2013, 7.5% of the total estimated corn harvested from 21 corn producing states was lost to disease; meaning nearly 27 million metric tons of corn was lost because of seedling blights and foliar diseases (Mueller and Wise, 2014). Under ideal weather conditions for pathogenesis, a 1% increase in foliar disease severity of Gray Leaf spot, caused by the fungus *Cercospora zeae-maydis*, reduced corn yields by 47.6 kg/ha when compared with a tolerant hybrid (Nutter and Jenco, 1992; Ward et al., 1999). Furthermore, in a meta-analysis of 20 studies, every 10% increase in rust severity on sweet corn, caused by the fungus *Puccinia sorghi*, reduced corn yields 2.4 to 7.0% (Shah and Dillard, 2006). Mycotoxins, a secondary metabolite of fungi, contaminated 12.5% of the total harvested grain in the United States in 2013; mostly because of the disease *Aspergillus* Ear Rot (Mueller and Wise, 2014), caused by the fungi *Aspergillus flavus* and *parasiticus* (Miller, 1995). Evidence shows fungal infection and disease on plants can cause devastating losses in corn yield.

Fungal Relationship with Plants

All fungal-plant relationships though are not parasitic. Most fungi associated with plants are saprotrophs, responsible for decomposing organic matter as their food source (Carris et al., 2012). Other fungi, about 160 known species, reside on the roots of growing plants in a mutualistic relationship. Carbohydrates produced by the plant feed the fungus, and the fungus transports nitrogen, phosphorous, and other minerals to the plant (Carris et al., 2012). A very small amount of fungi are disease causing, totaling less than 10% of about 100,000 known species, that colonize plants (Knogge, 1996).

Disease Triangle

Plant pathologists use the disease triangle for assistance when evaluating the likelihood of a disease outbreak. A susceptible host (plant), a pathogen, and a favorable environment are all necessary for development of plant infection, presence of just two is unlikely to result in disease. The relationship between fungi and plants is sometimes referred to as an ‘arm’s race’ (Malinovsky et al., 2014).

PATHOGEN: By definition, pathogens cause disease and need to complete their life cycle within the host (Sexton and Howlett, 2006). Historically, fungi can be divided into two main groups, both of which originate in the field. Field fungi, produce toxins in the plant before harvest and are governed by a plant-fungus interaction. Storage fungus is a problem postharvest, and a function of crop nutrients, physical, and biotic factors (Miller, 1995).

Most field fungi have a very narrow range of hosts, which can be further divided into a preferred plant maturity stage, e.g. seeds, seedling, or adult plants, and a preferred plant part, e.g. roots, leaves, stems, and fruits (Carris et al., 2012). In brief, field pathogenic fungi must germinate on the host’s surface, penetrate the host’s tissue, colonize within the tissue, alter the physiology of the plant cell, and cause disease (Sexton and Howlett, 2006). Fungal spores, known as conidia, allow for plant to plant transmission of fungal disease (Sexton and Howlett, 2006). Conidia can spread to infect other plants with the help of water droplets or wind (Sexton and Howlett, 2006) but can also spread as a result of previous harvest crop residues left in the field, which may harbor conidia and allow the fungus to travel up the plant for infection (Miller, 1995; Wise and Mueller, 2011).

Fungal spores germinate to form hypae, a filamentous structure which grow on the surface of the plant (Sexton and Howlett, 2006). Outside the plant cell, most fungi rely on the glyoxylate cycle, an anabolic pathway occurring in plants, bacteria, and fungi for conversion of acetyl-CoA to succinate, for nutrition until entry into the host.

Fungal pathogens enter into the plant cell either by natural opening or forced entry (Sexton and Howlett, 2006). Natural openings for fungal invasion may include the stomata (Sexton and Howlett, 2006), a pore on the leaf of plants allowing carbon dioxide to enter for photosynthesis (Freeman and Beattie, 2008), or bird, insect, or weather damage (Bradley and Ames, 2010) to the plant tissue allowing for fungal colonization (Miller, 1995). Forced entry into the plant cell may include the secretion of hydrolytic enzymes or mechanical force application (Sexton and Howlett, 2006). The secretion of enzymes generally include: cutinases to degrade the plant cuticle structural component cutin; cellulases to degrade the plant cell wall polysaccharide cellulose; pectinases to degrade the plant cell component pectin; and proteases (Knogge, 1996; Malinovsky et al., 2014). Development of a highly specialized fungal organ called apressoria in combination with invasive hyphae allow for direct access to the plant cell nutrients (Deising et al., 2000) by producing high turgor pressure and puncturing the plant tissue (Sexton and Howlett, 2006).

Once in the cell, the fungal pathogen needs to either adapt to the host's physiology or modify the environment for nutrient uptake to allow for colonization within the host (Knogge, 1994; Sexton and Howlett, 2006). Once fungal pathogens invade, plant cell oxidative bursts signal other metabolic pathways of an invasion (Malinovsky et al., 2014) but in doing so locally kill plant tissue providing immediate nutrients to the fungus (Sexton and Howlett, 2006). For more long term nutrition, a haustoria, a specialized fungal structure, can be inserted into the plant cell for water and nutrient uptake, especially hexose carbohydrates including sucrose, glucose and fructose (Voegele et al., 2001). The diversion of plant nutrients can be used for fungal growth and development.

Once inside the cell and growth has ceased, fungal pathogens release secondary metabolites, which in some species are toxic. It is generally hypothesized that during the colonization and sporulation phase of a fungus within a plant, mycotoxins are secreted by growing colonies (Calvo et al., 2002). The exact function of fungal toxins in the plant is unclear. Fungal phytotoxins can cause direct plant cell death (Sexon and Howlett, 2006) by over activation of the plant plasma membrane enzyme, H^{+}ATPase, which disrupts energy transfer during the light reactions in the chloroplasts (Knogge, 1996), the closing or opening of the stomata, and the redirection of ion channels (Elmore and Coaker, 2011)**.** But agriculturally, mycotoxins threaten food safety and security.

Five agriculturally important mycotoxins resulting from corn ear rot include: deoxynivalenol, from the fungus *Fusarium graminearum;* zeralenone from the fungus *F. graminearum*; ochratoxin A from the fungi *Piper verrucosum* and *A. ochraceus*; fumonisin from the fungus *F. moniliforme;* and aflatoxin from the fungi *A. flavus* and *A. parasiticus* (Miller, 1995). Development of mycotoxins within the plant occurs later in the growth and development of the corn plant. One study showed fumonisin concentration within corn kernels increased greatly as the corn plant became more mature, with only 33% of corn kernels infected at the fourth reproductive stage, but 62.5% of corn kernels infected at harvest (Ariño et al., 2009). Furthermore, while it is generally thought tilling fields may reduce fungi colonization it may not be the case as Ariño et al. (2009) showed no difference in fusonisin concentrations in varying degrees of tilled fields.

HOST: The plant's goal is to remain healthy and continue growing, but fungal invasions threaten the nutrient status of the plant. Therefore, defense mechanisms of plant cells include structural protection of the cell, detection of microbes, and chemical defense against invading organisms including fungi. The outermost protective tissue of the leaves and stems is covered in a waxy cuticle, known as the epidermis, which limits water loss and microbial infection (Freeman and Beattie, 2008). Furthermore, guard cells regulate the opening and close of the stomata on the leaf pores, thereby limiting the natural entry of fungus.

The cell wall is the next physical barrier to fungal invasion. The plant cell wall is composed of a primary cell wall, providing structural support for the plant, and a secondary cell wall, developing inside the primary cell wall only after the plant cells stop growing (Freeman and Beattie, 2008). The primary wall of plant cells is composed of cellulose, cross-linking glycans, also known as hemicellulose, and pectins. Cellulose is a polysaccharide, comprised of β (1,4)-glyosidic bonds between glucose molecules and very resistant to degradation by hydrolysis (Malinovsky et al., 2014). Hemicellulose is also a polysaccharide, where a pentose is bonded with a hexose, e.g. arabinoxylans, xyloglucans, mixed linked beta glucans, and galactomannans. The cross linking of hemicellulose aids in the fortification of cellulose for both structural support and prevention of microbial invasion (Malinovsky et al., 2014). Enzymes such as xylanase, produced by some fungi, weaken the cell wall and allow fungal entry into the plant cell (Malinovsky et al., 2014). Lignin, a phenolic polymer, is deposited during the last stages of secondary cell wall formation. Lignin reinforces plant cells and allows transport of water under negative cellular pressure (Malinovsky et al., 2014). When cell walls become lignified, it becomes highly impermeable to pathogens and hard for insects to digest, limiting access to cell wall sugars (Freeman and Beattie, 2008).

Plants evolved to quickly detect pathogens within the plant cell and rapidly respond to prevent serious damage. Plants do not have an immune system like animals; instead they have a recognition system controlled by resistance genes within the plant cell known as plant triggered immunity (PTI) (Sexton and Howlett, 2006). ‘Pathogen associated molecular patterns’, also known as PAMPs which may include fungal chitin or bacteria flagellin, can trigger a PTI response within the plant cell to prevent microbial colonization (Malinovsky et al., 2014). Also, ‘damage associated molecular patterns’, known as DAMPs which may include parts of the plant cell wall released possibly due to fungal enzymes, trigger an immune reaction (Malinovsky et al., 2014). An activated PTI in a plant cell may cause localized death (Malinovksy et al., 2014), an oxidative burst of reactive oxygen species to signal neighboring cells of invasion (Freeman and Beattie, 2008), a rapid fluctuation in the calcium gradient to signal that a pathogen has been detected (Malinovksy et al., 2014), release of pathogenesis related enzymes including chitinase, to degrade fungal chitin (Sexton and Howlett, 2006), activation of enzymes to strength the cell wall, activation of defense genes, and induction of phytoalexins, which are antimicrobial substance synthesized de novo (Knogge, 1996).

Lastly, plants produce primary and secondary metabolites. Primary metabolites are used in plant growth, reproduction, and development, while secondary metabolites are involved in plant defense (Freeman and Beattie, 2008). One class of secondary metabolites produced by plants is phenolic compounds including defense compounds such as: flavonoids, anthocyanins, phytoalexins, tannins, and lignin (Freeman and Beattie, 2008). Biotic and/or abiotic stresses can result in the increased lignin and phenolic content by inducing lignification of the walls that do not normally occur under non-stress conditions (Kim et al., 2007).

ENVIRONMENT: A favorable environment is needed for the development of plant disease, completing the final side of the disease triangle. The favorable environment for one species of fungi may be different for another species of fungi. For example, when growing conditions for corn include a warm ambient temperature and drought conditions, corn is more susceptible to the fungus *A. flavus* and *A. parasiticus*, which produce alflatoxin as a secondary metabolite (Richard, 2007). Yet, the foliar fungus *Exserohilum turcicum*, causing Northern Leaf Blight in corn, favors cool and humid conditions for colonization of foliage (Wise, 2011). Understanding the role the complex relationship between plant cells, fungi, and the environment is crucial for the future production of corn and those whom consume it.

Fungicides

Countries around the world seek to control fungal pathogens through various methods, including fungicide application on plants, in hopes that chemical application will alleviate their impact on corn. In keeping with the disease triangle, fungicide's aid in the plants defense from fungal invasion. The Food and Agricultural Organization estimated in 2013 that Brazil applied the most fungicide on crops, using 40 thousand tons of active ingredients, followed by Mexico and then Spain, using 38 thousand and 29 thousand tons of active ingredients, respectively (FAO, 2015b). Data for the United States in 2013 were unavailable, but in 2007 producers in the United States applied 20 thousand tons of active ingredients on crops (FAO, 2015b).

History

Within the last 50 yr, the development of modern fungicides and the split into different chemical classes based on how the chemicals inhibit fungal growth, including: triazoles, strobilurins, imidazoles, and pyrimidines, have allowed plant producers to protect crops, such as corn, from fungal pathogens. Because different classes of fungicides control various stages of fungal development, fungicide classes are spilt into three groups: 1. controlling pathogens before entry into the host, 2. control during colonization, and 3. control during the final reproductive stages of development (Hewitt, 2000).

Classes of fungicides and mode of action

Strobilurins fungicides, also known as QoI fungicides, are natural chemical structures isolated from the genera *Strobilurus*, in wood-rotting mushrooms. Since natural strobilurins break down quickly in UV light, synthetic analogs were developed for disease control (Balba, 2007). Strobilurin fungicides are broad-spectrum fungicides, meaning the fungicide controls an wide array of fungal diseases in a variety of crops including cereals, fruits, vegetables, tree nuts, turf grasses, and ornamentals (Vincelli, 2002). Within the strobilurn class of fungicides is the active ingredient pyraclostrobin, which is used in the commercial products Headline AMP (BASF Corp®, North Carolina) and Priaxor (BASF Corp®, North Carolina). Also included in the strobilurin class of fungicides are products known as: Quadris® (Sygenta, North Carolina), Hertiage® (Sygenta, North Carolina), Zolera FX™ (Arysta, North Carolina), and Disarm 480 SC™ (Arysta, North Carolina). How the fungicide inhibits the fungus, also known as mode of action, is target specific. Strobilurins bind to the quinol oxidation (Qo) site of cytochrome b. This binding stops the electron transport between cytochrome b and cytochrome c, stopping the oxidation of nicotinamide adenine dinucleotide (NADH) and synthesis of adenosine triphosphate (ATP). Effectively, strobilurins stop energy production and kill the fungus. Application of strobilurin on plants rapidly act on spore stage of fungal development, and because of this are described as giving the plant a “greening effect”, which is beneficial to the plant by adding more green color (Balba, 2007). Once on the waxy leaf surface, strobilurins move throughout the plant either translaminarly and/or systemically (Vincelli, 2002). Translaminar movement occurs when fungicide affinity for the waxy cuticle holds the fungicide to one side of the leaf blade, but some ‘leaks’ through to the far side cuticle, allowing for fungicide affinity to hold it present on both sides of the leaf even though application only occurred on one. Systemic movement occurs when the fungicide moves as a gas in the layer of air adjacent to the leaf, known as the boundary layer (Vincelli, 2002). Although, most strobilurin fungicides are weak and only locally systemic in movement across leaf blades, resulting in high effectiveness early in spore development, but once the fungus is in the tissue, relatively ineffective (Balba, 2007). Lastly, because the fungicide acts on a specific site, only one mutation is needed to create a fungicide resistance fungus, a concern for plant pathologists.

A second group of fungicide commonly used today is carboxamide fungicides, also referred to as succinate dehydrogenase inhibitors (SDHI). Within the SDHI class of fungicides is the active ingredient fluxapyroxad, included in commercial products such as Prixaor (BASF, Corp®). Other fungicides within the SDHI class include: Serguris Flexi® (Sygenta, North Carolina) and Vitaflo® (Chemtura, Pennsylvania) Like the strobilurins, the class is very diverse in active ingredients, but all chemicals within SDHI class contain an amide bond connected to a five or six ring structure (Sierotzki and Scalliet, 2013). Succinate dehydrogenase inhibitors are broad-spectrum fungicides and can have translaminar or systemic activity within the host, depending on the pathogen and host (McKay et al., 2011). Furthermore, SDHI are site-specific fungicides, targeting the succinate dehydrogenase complex in the respiratory chain, known as complex II (Avenot and Michailides, 2010). By blocking the ubiquinone binding sites, the fungicides inhibit fungal respiration (Avenot and Michailides, 2010; McKay et al., 2011). Because SDHI are site specific, prevention of fungal resistance is crucial to allow for the product to continue working on pathogens.

A third group of fungicide is known as the demethylation inhibitors (DMIh) or sterol biosynthesis inhibitors (SBIs), which contain the triazole fungicides. Within the triazole class, is the active ingredient metconazole, included in commercial products such as Headline AMP (BASF, Corp®). Other fungicides including a triazole active ingredient include Stratego (Bayer, New Jersey), Banner MAXX® (Sygenta, North Carolina), and Spectator™ (Lesco, Inc., Ohio). Demethylation inhibitor fungicides are systemic (Lepeseheva and Waterman, 2007) and single-site specific inhibitors commonly used on cereal grain (Lucas et al., 2015). Fungicide active agents act on the membrane biosynthesis process, targeting the protein sterol known as CYP51, which belongs to the cytochrome P450 monooxygenase superfamily (Becher and Wirsel, 2012). Cytochromes P450 are proteins found in all biological kingdoms and catalyze mono-oxygentations, which are the addition of a hydroxyl into metabolism. In fungi, CYP51 have a narrow function involved in removing the 14-methyl group of sterol precursors (Becher and Wirsel, 2012) and forming ergosterols (Lepeseheva and Waterman, 2007). Ergosterols are a part of the plasma membrane regulating membrane fluidity and permeability in fungi (Lepeseheva and Waterman, 2007). Therefore, using DMIh fungicides block sterol biosynthesis impacting fungal growth and development. Just as with QoI fungicides and SDHI fungicides, repeated use of DMIh is cautioned as fungi can just develop one mutation to be resistant to this class of fungicide.

Fungicide modes of action are highly specific in the species of fungi they inhibit and how the chemicals act within the fungi. Modern fungicides include more than one mode of action within the fungal treatment to ensure the fungus is knocked out even if some resistance has developed.

Benefits of fungicide application

In recent years, some researchers and chemical companies have concluded foliar fungicide application on corn may increase yields even in the absence of disease (Wise and Mueller, 2011). In a meta-analysis on yield response and pyraclostrobin fungicide treatment, the mean difference in yield for plots treated with foliar fungicide increased 255.91 kg/ha compared with untreated plots (Paul et al., 2011). Yet, even so some researchers are not entirely convinced applications increase yields the same in every field. Paul et al. (2011) concluded that when disease in the field is < 5% the likelihood of an advantageous yield bump and beneficial physiological response enough to cover the cost of applying the fungicide is not as likely. But when disease in the field is > 5%, fungicide application is more helpful by limiting yield losses due to fungal infection. Furthermore, in a consecutive two-year study Bradley and Ames (2010) did not see an increase in yield in 2008, under low disease severity environments, but in 2007 did see a yield increase when under higher disease severity. Routine scouting for disease in the cornfield is crucial for determining when fungicide application will be most profitable.

When a producer's field is diseased, proper timing of fungicide application on the plant may also provide beneficial results. Under pressure from fungal disease, application of pyraclostrobin on corn at VT (vegetative stage tassel) increased yield by 550 kg/ha compared to untreated fields of corn (Nelson and Meinhardt, 2011). But others have shown earlier applications to be beneficial as well. In a year with high incidence of common rust, foliar fungicide applied as a preventative at vegetative stage six (V6), when six leaf collars are visible on the growing plant (Mueller and Pope, 2009), increased corn grain yield by 362.9 kg/ha compared to application at pre-tassel, when 6% of the total leaf area was diseased (Wright et al., 2014). Yet, in a different year of the same study, when disease incidence was low, foliar fungicide applied as a preventative at V6 did not increase corn grain yield when compared to application at tassel (Wright et al., 2014).

Lastly, fungicide applications on corn may increase the concentration of nutrients within the plant material. In 2007, the University of Wisconsin reported a 1.9 percentage unit increase in starch concentration and 1 percentage unit decrease in NDF concentration when comparing corn silage from corn treated with foliar fungicide application, compared to untreated corn (Blonde and Esker, 2008).

Mycotoxin and fungicides

Fungicides have been tested for preventing fungal colonization and mycotoxin contamination in cereal grains. Results of studies have been conflicting in their ability to control mycotoxin concentration within crops. Applications of metconazole and tebuconazole, another active ingredient of fungicide, reduced concentrations of DON and head blight in winter wheat more than applications of azoxystrobin, another active ingredient (Edwards et al., 2001). But some researchers hypothesize fungicides act as an additional stress factor for the fungus and stimulate mycotoxins as a defense mechanism (Magan et al., 2002).

Variation in results

The efficacy of foliar fungicides applied on corn hybrids is variable, subject to: timing of application (Paul et al., 2011; van den Berg et al., 2013), weather conditions (Mueller and Pope, 2009; Paul et al., 2011; Wise and Mueller, 2011), disease pressure (Mueller and Pope, 2009; Munkvold et al., 2001; Paul et al., 2011), planting date (Wise and Mueller, 2011), spray doses (van den Berg et al., 2013), number of sprays (Munkvold et al., 2001), mode of action of active ingredients in the fungicide (Paul et al., 2011; Wise and Mueller, 2011), history of the field (Mueller and Pope, 2009; Munkvold et al., 2001), agronomic practices (Munkvold et al. 2001;

Paul et al., 2011), fungus resistance to fungicide (Munkvold et al., 2001; Wise and Mueller, 2011), and hybrid seed disease resistance (Munkvold et al., 2001; Mueller and Pope, 2009; Paul et al., 2011).

Cost-benefit relationships

Application of fungicide to assist in fungal control on corn costs producers money. But some of value may be returned to producers by increasing the efficiency of converting feed to milk when feeding feedstuffs with fungicide application in the field to dairy. During the 2014 growing season, corn was sprayed with foliar fungicide either once, twice, three times or not at all and ensiled as corn silage. Then during the summer months of 2015, dairy cattle were fed corn silage from corn treated with foliar fungicide to evaluate the effects on milk production and efficiency (Haerr et al., 2015). The section below will discuss more about the dairy cow and digestive system, but in an economic analysis the total income from milk yield over feed costs in 2015 was $7.35, $7.54, $8.31, and $7.83 for CON, one application, two applications, or three applications of fungicide, respectively (Haerr, 2015). Therefore, it seems cows fed corn silage from corn with fungicide treatment are more profitable than cows fed corn silage with no treatment.

Corn as a feedstuff

In 2010, 43% of U.S. corn was used for livestock and poultry diets, 42% was used for ethanol production, and 11% used for food (NASS, 2010). Mycotoxins, the secondary metabolites formed from fungus, can cause disease in animals if consumed in too great of concentrations. For example, deoxynivalenol, from the fungus *F. graminearum,* can cause acute toxicosis in swine, manifested through intestinal disorders and vomiting (Miller, 1995). Furthermore, aflatoxin, from the fungus *A. flavus* and *parasiticus*, consumed by cattle resulted in weight loss and decreased milk production (Miller and Wilson, 1994). Additionally, threatening human food security, alfatoxin can be found in the milk of dairy cattle as M1 (Richard, 2007) and is toxic to humans. In recent years, crop scientists, microbiologists, and animal nutritionists have sought to develop solutions to reduce the impact of fungi on feed for animals and limit the concentration of toxins in products for human consumption.

Dairy cattle and corn silage

In 2013, India, Brazil, and the former Sudan had the largest population of dairy cattle with 45 million, 23 million, and 15 million, respectively (FAO, 2015c). The United States ranked 8th in population with an estimated 9 million dairy cattle. However, total milk yield was greatest for the United States (81 million tons), followed by India (55 million tons), and China (33 million tons) (FAO, 2015c). Improvements in how dairy cattle are fed may help explain why the United States produced milk more efficiently compared to others.

In the United States, corn silage is one of the most popular forages fed to ruminants. The USDA reported 14% of all corn harvested in 2014 was for corn silage production and 89.4% of dairy operations in the United States included corn silage in the lactating diet (USDA, 2014). It is important to remember that corn silage is heterogeneous combination of fiber and starch from various parts of the corn plant: including stalks, leaves, cob and kernels. On a dry matter basis, whole plant corn silage is composed of about 57% corn ears, 13% corn leaves, and 31% corn stems (Kuehn et al., 1999).

Ensiling corn

At time of harvest, dairy producers store and preserve corn material as silage, which can be fed all year. The process of ensiling corn is broken down into four phases with varying lengths of time. The first phase, the aerobic period, is characterized by the reduction of atmospheric O_2 within a couple hours postharvest, meanwhile active proteases decompose proteins and carbohydrates to amino acids and soluble carbohydrates. The second phase, the fermentation phase, anaerobic microorganisms compete with one another for nutrients, and in well fermented silages, lactic acid bacteria (LAB) eventually dominate lowering the pH (Pahlow et al., 2003). The third phase, the stable phase, continues with the slow hydrolysis of structural and storage carbohydrates, and if air is properly excluded can last any length of time. The fourth phase, the feed out phase, is where plant material is exposed to O_2 causing aerobic organisms to develop (Pahlow et al., 2003).

Fungi can also attack the plant material in storage. To limit the growth and colonization, generally, it is recommended to store corn material in dry conditions and as mature crops (Richard, 2007). The occurrence of fungi in silages usually is the result of poor sealing and poor compaction causing aerobic conditions in the silo, not only causing losses of feed, but also reductions in palatability (Pahlow et al., 2003). Furthermore, visibly molded areas of silages underestimate the amount of fungi within the silage content, as well as the high probability of mycotoxins (Pahlow et al., 2003). More exists than visible by the human eye.

Under proper forage management, corn material from the previous season's harvest should be enough to feed for the year, until the new harvested silage has undergone all four phases. Nevertheless, this is not always the case and producers need to feed silage as soon as possible. The length of ensiling has been shown to have significant effects on the nutritional content of the feedstuff including: dry matter (Der Bedrosian et al., 2012; Weinberg and Chen, 2013), lactic acid concentration (Ferraretto et al., 2015) acetic acid concentration (Der Bedrosian et al., 2012; Weinberg and Chen, 2013; Ferraretto et al., 2015), neutral detergent fiber digestibility (Der Bedrosian et al., 2012; Weinberg and Chen, 2013), and concentration of crude protein (Der Bedrosian et al., 2012).

Dairy diet and limitations

Corn silage represents about 40 to 60% of the total mixed ration in the lactating diet. Dry matter intake and energy concentration of corn silage determine the energy intake, and therefore, the cow's performance (Allen et al., 2003). Ruminant forage diets are limited by the amount of fiber within the plant material (Van Soest, 1994). The proximate analysis partitions compounds within feed into six categories, including crude fiber. According to Van Soest (1994), the proximate analysis method was outdated, therefore, the detergent system was developed as a rapid procedure to determine the concentration of insoluble cell wall within a feedstuff, by estimating the major subcomponents including: hemicellulose, cellulose, and lignin (Van Soest, 1994). Neutral detergent fiber, NDF, is a laboratory procedure that can be performed to estimate the amount of cellulose, lignin, and hemicellulose within a sample. Acid detergent fiber, ADF, is also a laboratory procedure that can be performed to estimate the amount of cellulose and lignin within the sample. Van Soest (1965) found that NDF is highly correlated with dry matter intake; the higher the concentration of NDF within the diet, the lower the DMI, partially as a result of rumen fill and digestibility.

Greater lignification of plant cell walls may increase bulk density of the feedstuff or require greater energy concentration of the diet to meet the nutritional needs (Allen et al., 2003). Increased lignin concentration within the plant cell has been thought to be the primary limitation to cell wall digestibility. Intense lignification creates an absolute barrier for rumen bacteria when digesting one plant cell wall and moving to the next cell (Jung, 2012). Mechanical chopping of plant material and cow chewing assist in creating small tears in the silage allowing rumen microbes and enzymes access to degrade the feedstuff, but even under these conditions, the concentration of lignin within the plant material does not change.

Therefore, techniques to alter the fiber content within the silage may create a more digestible feedstuff for dairy cows and impact milk production. An analysis of 20 experiments reported increasing NDF content (Mean: 36.87 ± 5.81% of DM; Min: 22.30% of DM; Max: 51.60% of DM) of corn silage fed to dairy cattle was negatively associated with lower milk yield (R^2 = 92.1), and lower FCM (R^2 = 88.0) (Briceno et al., 1987). Furthermore, dry matter intake and milk yield decreased for cows fed diets containing increased concentrations of NDF, ADF, and lignin and decreased fiber digestibility (Oba and Allen, 1999). In an analysis of 162 treatments, DMI and milk yield were 0.7 kg/d and 1.0 kg/d greater, respectively, for cows fed corn silage with high *in-vitro* digestibility compared to a conventional corn silage (Ferraretto and Shaver, 2015). Furthermore, in a meta-analysis, 1 percentage unit increase in NDF digestibility, measure *in vitro* or *in situ*, resulted in 0.25-kg increase in fat corrected milk (Oba and Allen, 1999). Reducing the amount of fiber present in the cell wall can have positive benefits in terms of production for dairy producers.

Corn silage quality

Nutritionists, producers and veterinarians evaluate corn silage quality when feeding to dairy cattle, as it directly relates to energy intake and milk production. Laboratory procedures and on farm tests allow producers to evaluate the diet quickly and make the necessary updates to the diet.

In a laboratory, various wet chemistry tests can be performed on feedstuffs to evaluate the concentration of fiber, proteins, sugars, and fats. Using the NRC (2001), a variety of calculations can be determined including net energy of lactation, as well as, fat-corrected milk and feed conversion values. Also, an analysis of the kernel processing (also referred to as corn processing score in some labs) evaluates how well the kernels have been mechanically damage. Shaking a set of ten sieves for 10 min, the kernel processing score is calculated as the total starch in the sample minus the starch that does not pass through the 4.75-mm sieve, equaling the amount of starch passing through the 4.75-mm sieve. Results of tests where percentage of starch is greater than 70% are interpreted as optimum processing of kernels, 50 to 70% as adequate processing and less than 50% inadequately processed (Dairy One, 2015a). Kernel processing corn at 1mm when harvesting, ensiled as corn silage and fed to cows tended to decrease the concentration of starch excreted (336 g/d) in the feces compared with unprocessed corn (442 g/d) (Dhiman et al., 2000). In a separate experiment, kernel processing improved total starch digestibility (87.4%) compared to unprocessed (84.3%) (Dhiman et al., 2000).

Results from other tests aid producers in making dietary adjustments including: a Penn State test, a density test, and an aerobic stability test. A Penn State test may be used on both TMR and corn silage to evaluate the distribution of particle size using 4 sieves with pore sizes: 1.9 cm, 0.8 cm, 0.1 cm and a pan. The upper sieve is for particle sizes greater than 0.13 cm, the middle sieve holds particles 0.13 and 0.79 cm, the bottom sieve holds particles 0.07 to 0.13 cm, and the pan catches the rest of the particles. Penn State tests can be performed on TMR or corn silage. For TMR, guidelines for the percentage of feed in each of the sieves is 2-8% in the upper sieve, 30 to 50% in the middle sieve, 30 to 50% in the lower sieve, and less than or equal to 20 for the bottom pan (Heinrichs and Kononoff, 2002). For corn silage, guidelines for the percentage of feed in each of the sieve is 3 to 8% in the upper sieve, 45 to 65% in the middle sieve, 30 to 40% in the lower sieve, and less than 5 in the bottom pan (Heinrichs and Kononoff, 2002). Numbers outside the guidelines may be indicators of inadequate forage particle length necessary for rumen function, and can help trouble shoot metabolic or feeding problems.

Drilling the density probe into the silo, the as-fed density and dry matter density can be calculated and used to evaluate the packing of the silo (Dairy One, 2015b). Muck and Holmes (1999) determined the minimum packing DM density of corn silage to be 225 kg/m^3. Data from a collection from samples shows the average 233 kg/m^3, with a range of 125 to 405 kg/m^3 (Dairy One, 2015b). Interpretation from density results can offer insight into packing density and how to improve silage management in the future for better fermentation should numbers appear outside the recommendations.

An aerobic stability test of corn silage is the amount of time required to raise the temperature two degrees above the ambient temperature when exposed to air. When corn silage is first exposed to air, silage deteriorates as a result of aerobic microbial activity. Kernel processing corn ensiled as corn silage tended to increase the aerobic stability of corn silage (57.6 h), measured as the number of hours to reach 1.7°C above ambient temperature, compared to unprocessed corn silage (44.1 h) (Johnson et al., 2002). Because dairy producers cannot directly control the quality of corn silage available, these tests allow nutritionists and producers to make the necessary adjustment to ensure a balanced, energy rich diet.

Lastly, an *in situ* estimation of digestibility in the rumen is a valuable tool to estimate the nutritional value of feedstuffs (Van Milgen et al., 1991), as it gives an estimation of the rate of degradability within the living animal and allows inferences to be made about the nutritive quality of the feedstuff. Suspending polyester or nylon bags filled with feedstuffs in the rumen of cannulated dairy cows allows for the determination of the proportion remaining at various times after incubation (Cherney and Cherney, 2003). Although, differences in techniques, materials, and lack of standardization between experiments can make interpretation and relation of results difficult (Vanzant et al., 1998).

Diseased corn silage

From the previous discussion, it is no surprise fungal disease on corn, ensiled as corn silage can impact the nutritional content within the plant material. Inoculation of Northern Leaf Blight, caused by the fungus *Exserohilum turcicum,* on corn increased the NDF and ADF concentration 52.6 g/kg of DM and 41.2 g/kg of DM, respectively, compared to non-diseased corn (Wang et al., 2010). The corn was then ensiled as corn silage and fed to sheep. Corn silage from diseased corn resulted in a greater concentration of NDF (499.9 ± 40.1 g/kg of DM) and ADF (263.0 ± 32.5 g/kg of DM) when compared to corn silage from non-diseased corn (392.1 ± 32.1 g/kg of DM and 217.0 ± 30.3 g/kg DM for NDF and ADF, respectively) (Wang et al., 2010). Dry matter digestibility was less for sheep consuming corn silage from diseased corn (0.665 ± 0.029) compared to control (0.725 ± 0.012), measured using metabolic crates (Wang et al., 2010). Yet, dry matter intake was not different for sheep consuming corn silage from diseased corn (34.6 ± 4.1 g/kg of $BW^{0.75}$/d) compared to control (40.9 ± 4.1 g/kg of $BW^{0.75}$/d) (Wang et al., 2010).

In another study, corn was inoculated with either no fungi, a medium concentration or a high concentration of Southern Rust, caused by the fungus *Puccinia polysora,* and then, ensiled as corn silage. Increasing the rust infestation from no rust to medium rust to high rust concentration on corn ensiled as corn silage increased the DM concentration, the concentration of NDF (no rust: 44.1% of DM, medium rust: 47.7% of DM, and high rust: 48.5% of DM) and ADF (no rust: 23.1% of DM, medium rust: 25.1% of DM, and high rust: 25.3% of DM), and decreased the *in vitro* DM true digestibility (no rust: 66.9%, medium rust: 63.2%, and high rust:

60.1%) and *in vitro* NDF digestibility (no rust: 38.1%, medium rust: 39.8%, and high rust: 36.2%) (Queiroz et al., 2012). Additionally, increased rust infestation on corn silage resulted in worse fermentation conditions exhibited by: increased pH (no rust: 3.65, medium rust: 3.71, and high rust: 3.97) and decreased lactate (no rust: 4.99, medium rust: 4.02, and high rust: 2.28%). Aflatoxin was detected in corn silage from corn with a high concentration of Southern Rust at a concentration of 5.20 mg/kg of DM (Queiroz et al., 2012). Zearalenone was detected only in corn silage with no concentration of Southern Rust at a concentration of 0.64 mg/kg of DM (Queiroz et al., 2012).

Another set of researchers evaluated physically damaging the ears of corn in the field prior to harvest on the production of mycotoxins and fermentation when ensiled as corn silage, to represent insect or hail damage on corn. In the first experiment, physical damage to corn kernels occurred at the milk stage of corn development (R3) slashing a knife through the kernels. Corn from experiment one was ensiled as corn silage for 126 d. Physical damage to the corn ear resulted in an increased concentration of fumonisin B1 (8.50 mg/kg for damaged and 4.00 mg/kg for undamaged DON (3.12 mg/kg for damaged and 0.92 mg/kg for undamaged) but decreased the concentration of zearalenone (1.03 mg/kg for damaged and 0.46 mg/kg for undamaged) in corn silage (Teller et al., 2012). Neutral detergent fiber and ADF was not different for corn silage physically damaged (45.0 and 26.8% of DM for NDF and ADF, respectively) compared with undamaged (45.2 and 27.3% of DM for NDF and ADF, respectively). In experiment two, physical damage to the corn kernels occurred either 27 d or 9 d prior to harvest, and was ensiled for 95 d. Damage to corn kernels 27 d prior (29.5% of DM) to harvest resulted in an increased ADF content in corn silage compared to 9 d prior (25.2% of DM) or no damage (25.7% of DM) (Teller et al., 2012). Corn silage damaged 27 d prior to harvest resulted in an increased concentration of ADF (31.9% of DM) and NDF (48% of DM) when compared to corn silage from non-damaged ears (22.3 and 36.3% of DM for ADF and NDF, respectively) (Teller et al., 2012). Furthermore, corn silage from corn damaged 27 d prior to harvest resulted in an increased concentration of DON (14.77 mg/kg), fumonisin B1 (7.63 mg/kg), and zearalenone (3.66 mg/kg) when compared with corn silage from undamaged corn kernels (0.18, 1.03, and 0.99 mg/kg for DON, fumonisin B1, and zearalenone, respectively) (Teller et al., 2012).

Fungicide on corn ensiled as corn silage

Researchers at the University of Wisconsin applied pyraclostrobin on corn and ensiled it as corn silage. Using the MILK 2006 model, pyraclostrobin application on corn numerically increased projected milk production by 75 lbs milk/ton DM (37 kg milk/ metric ton DM) when compared with control (Blonde and Esker, 2008).

As previously mentioned, Haerr et al. (2015) fed cows corn silage from corn with either one application of foliar fungicide, two applications of foliar fungicide, three applications of foliar fungicide, or no application of foliar fungicide. A decreasing linear relationship was reported for the number of fungicide applications and DMI (23.8, 23.0, 19.5, and 21.3 kg for CON, 1x, 2x, and 3x, respectively) but constant milk production among treatments (34.5, 34.5, 34.2, and 34.3 kg/d, for CON, 1x, 2x, and 3x, respectively) (Haerr et al., 2015). Therefore, cows fed corn silage from corn treated with foliar fungicide tended to have better-feed conversion milk yield/DMI values (1.46, 1.47, 1.70, and 1.70 kg/kg, for CON, 1x, 2x, and 3x, respectively), 3.5% FCM values (1.47, 1.51, 1.71, and 1.73, for CON, 1x, 2x, and 3x, respectively) and ECM valued (1.43, 1.46, 1.66, and 1.69 for CON, 1x, 2x, and 3x, respectively) (Haerr et al., 2015). The authors hypothesized that improved feed efficiency occurred because corn silage from corn treated with foliar fungicide application may have had an increased nutritive quality compared to untreated corn silage.

Conclusions and Objectives

The use of foliar fungicide on corn, ensiled as corn silage may have the potential to increase milk production and improve the feed conversion of feed to milk. The field of knowledge of feeding cows corn silage from corn treated with foliar fungicide is still narrow, but findings from previous research highlight the negatives of making and feeding silage from diseased corn plants. Fungicide application on corn used to make corn silage may reduce the fiber concentration, improve the digestibility, improve nutritive value, decrease mycotoxins, and improve cow health. Thus the objectives of the following studies were to examine the timing of application of foliar fungicide on corn:

1) Ensiled as corn silage and its effect on cow milk production, milk components, health, and intake;
2) Ensiled as corn silage on the *in situ* digestibility of corn silage;
3) Ensiled as corn silage on the nutrient quality, aerobic stability, thermal imaging, and density of corn silage;
4) On the nutritive quality of stems, leaves, ears, and flag leaves, individually;
5) On the fermentation and nutritive quality of corn silage using laboratory scale silos.

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TABLES AND FIGURES:

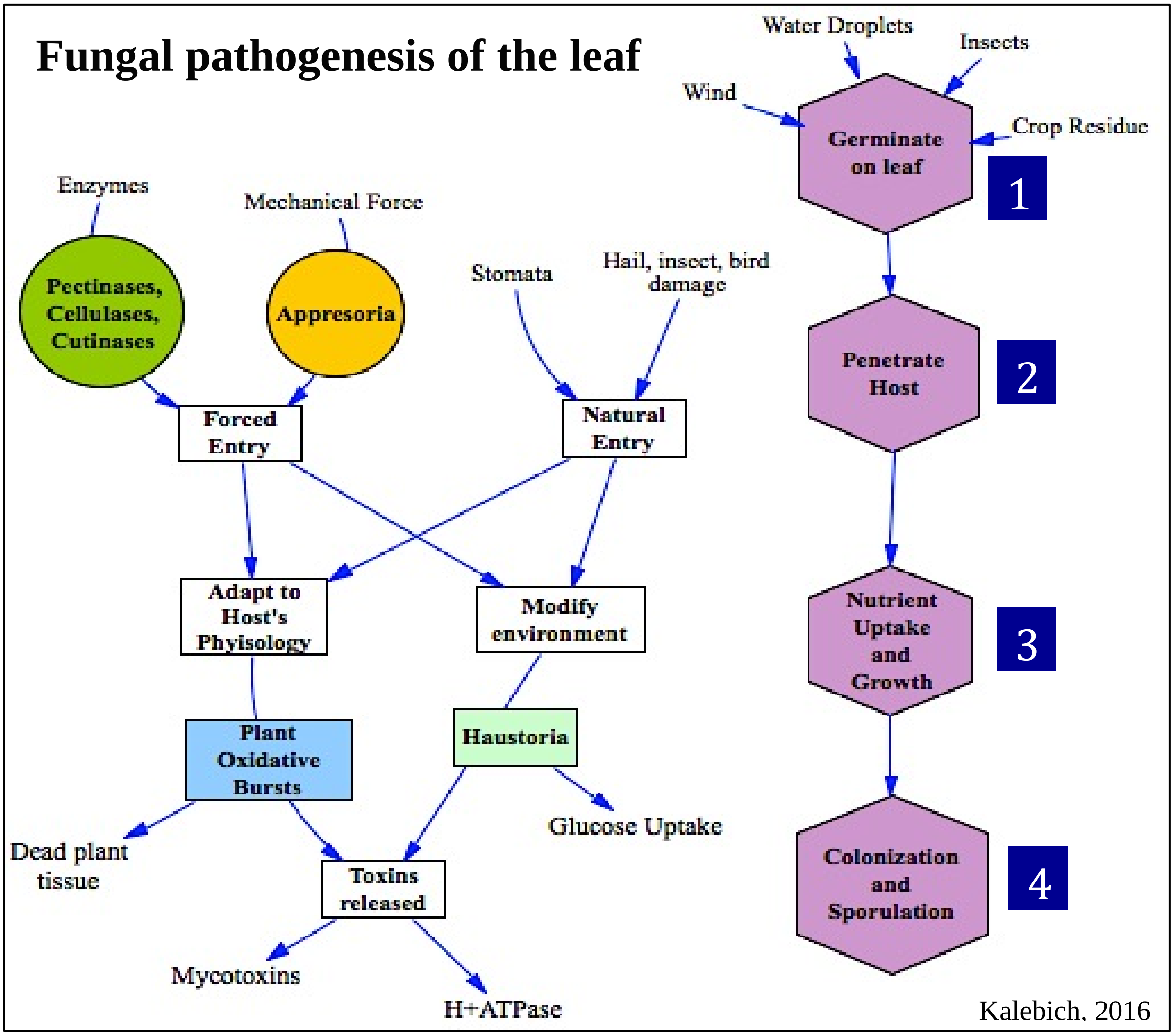

Figure 1.1: Fungal pathogenesis of the leaf

(1) **Germinate on leaf** - Spores must first be transported to the leaf, which can occur by the wind, water droplets, insects, or leftover crop residues from the previous harvest. Once one the leaf, the spores begin to grow and develop until a greater source of nutrition is needed.

(2) **Penetrate Host** – Fungi penetrate the plant tissue in either 1 of 2 ways (forced or natural entry), depending on the species. In a forced entry, fungi use enzymes including: pectinases, cellulases, or cutinases to degrade the plant surface or fungi insert a hyphal organ known as appresoria into the host, causing high turgor pressure. In a natural entry, fungi enter the plant cell either through the stomata or through holes that have been created due to hail, insect or bird damage.

(3) **Nutrient Uptake and Growth** – Once inside the plant tissue, fungi can either adapt to the host's physiology or modify the environment for nutrient uptake to help facilitate further growth. When the fungi adapt the plant's physiology, fungi consume dead plant tissue as a result of stressed induced oxidative bursts. When the fungi modify the environment, fungi insert a slender portion of the hyphae into the cell to consumed hexoses, more specifically, glucose.

(4) **Colonization and Sporulation** – After the fungi has matured, the final stage is to reproduce and colonize the tissue. One example of the toxins released by some species is mycotoxins. It is hypothesized one of the targets of fungal toxins is the plant enzyme H+ATPase. In the plant, this enzyme is necessary for ion and metabolite transport.

Image adapted from: Knogge, 1994; Voegele et al., 2001; Calvo et al., 2002; Sexton and Howlett, 2006; Freeman and Beattie, 2008; Malinovsky et al., 2014.

CHAPTER II

Dry matter intake, milk yield, and milk composition of Holstein cows fed corn silage with various application times of foliar fungicide on the growing plant

ABSTRACT

Foliar fungicide application on corn plants at various times can manage fungal disease when growing in the field, but little is known about how the timing of fungicide application affects corn silage when fed to dairy cattle. The objective of this study was to determine which fungicide application time on corn, and ensiled as corn silage, would have the most advantageous impact on milk yield and composition in dairy cattle. Holstein cows (n = 64) with 2.2 ± 0.8 parity, 626 ± 77 kg of body weight, and 134 ± 37 d in milk were blocked and randomly assigned to 1 of 4 treatments (45% of the dietary DM as corn silage). Treatments were as follows: corn silage with no application of foliar fungicide **(CON)**; corn silage received one application of pyraclostrobin and fluxapyroxad (PYR+FLUX) foliar fungicide (Priaxor®; BASF Corp.) at corn stage V5 **(V5)**; corn silage received one application of PYR+FLUX at corn stage V5 plus another application of PYR+FLUX at corn stage V8 **(V5/V8)**; corn silage received one application of PYR+FLUX at corn stage V5, one application of PYR+FLUX at corn stage V8, plus a third application of pyraclostrobin and metconazole (PYR+MET) foliar fungicide (Headline AMP®; BASF Corp) at corn stage R1 **(V5/V8/R1)**. Corn was harvested at 31.2% DM and ensiled for more than 200 d. Treatments were fed to cows for 5 wk with only the last wk being used for statistical inferences. Three contrast statements were used: contrast 1: CON vs. TRT compares control to the average of treatments fed silage from corn sprayed with foliar fungicide (V5, V5/V8, and V5/V8/R1); Contrast 2: V5 vs. V5/V8 compares the treatment fed silage from corn

sprayed at V5 to the treatment fed silage from corn sprayed at V5 and V8; and contrast 3: V5/V8 vs. V5/V8/R1 compares the treatment fed silage from corn sprayed at V5 and V8 to the treatment fed silage from corn sprayed at V5, V8, and R1. No differences in DMI (19.5, 19.5, 20.8, and 20.4 kg for CON, V5, V5/V8, and V5/V8/R1, respectively) or milk yield (30.55, 31.17, 29.06, and 29.33 kg/d) were observed. However, cows in V5 when compared with cows in V5/V8 tended to produce more3.5% fat corrected milk (FCM; 32.42 and 28.58 kg/d, respectively) and energy corrected milk (ECM; 31.35 and 27.76 kg/d, respectively). Concentration of milk lactose tended to be greater for cows fed corn silage treated with foliar fungicide when compared with CON. In conclusion, cows in V5 tended to have greater FCM and ECM than cows in V5/V8.
Key words: corn silage, foliar fungicide, fungus, fat-corrected milk, lactating cattle

INTRODUCTION

In 2014 of the total 33 million ha of corn harvested, 2.6 million ha were harvested for corn silage (7.8%; USDA, 2015). Corn silage is a popular forage fed to dairy cattle where the climate is well adapted for corn growth (Allen et al., 2003). Because of the advantageous nutritional content of heterogeneous silage and the ease of handling and incorporating it into the diet (Blasel et al., 2006), the use of silage is positively correlated with an increase in herd size (Cherney and Cherney, 2003).

Fungal growth on corn in the field directly affects the nutritional quality of the feedstuff in terms of altered sugar concentration and fibrous content. Corn silage from corn infected with the fungal disease Northern Leaf Blight had increased NDF and decreased water-soluble carbohydrates content, reducing total DM digestibility when fed to sheep (Wang et al., 2010). When the corn plant is stressed with disease, less sugar is photosynthesized. Often, a lower concentration of sugar within the plant results in a lower quality stalk, as the sugar available completes the grain fill process in the corn ear (Nafziger, 2012); fields infected with Northern Leaf Blight resulted in premature stalk death and stalk lodging (Lipps, 1998).

Recommendations to reduce tillage, for preservation of the top layer of soil (Wise and Mueller, 2011), have increased foliar corn diseases such as Gray Leaf Spot, Northern Leaf Blight, and common rust attributed to decomposing residues from the previous harvest (Lipps, 1998). Although approved for use in the 1990s, it was not until the mid-2000s when the use of foliar fungicides was adopted into management practice (Wise and Mueller, 2011). Adoption of fungicide application was slow because of variability in profitability attributed to price of application, seed hybrid resistance, and disease level (Munkvold et al., 2001). A combination of research results and economic market conditions encouraged crop producers to embrace fungicide use on crops, even in the absence of disease pressure for improved plant health (Paul et al., 2011; Wise and Mueller, 2011; Bradley, 2012). In a meta-analysis evaluating corn yields in response to pyraclostrobin fungicide treatment, a mean increase of 255.91kg/ha in corn yields for fields treated with fungicide compared with untreated fields was reported (Paul et al., 2011). Furthermore, a study conducted by the University of Wisconsin in 2007 reported a 1.9 percentage unit increase in starch concentration and a 1 percentage unit decrease in NDF concentration when comparing foliar fungicide treated corn silage to untreated corn silage (Blonde and Esker, 2008). Yet, the efficacy of foliar fungicides applied on corn hybrids is variable subject to timing of application (Paul et al., 2011; van den Berg et al., 2013), weather conditions (Mueller and Pope, 2009; Paul et al., 2011; Wise and Mueller, 2011), disease pressure (Mueller and Pope, 2009; Munkvold et al., 2001; Paul et al., 2011), planting date (Wise and Mueller, 2011), spray doses (van den Berg et al., 2013), number of sprays (Munkvold et al., 2001), mode of action of active ingredients in the fungicide (Paul et al., 2011; Wise and Mueller, 2011), history of the field (Mueller and Pope, 2009; Munkvold et al., 2001), agronomic practices (Munkvold et al. 2001; Paul et al., 2011), fungus resistance to fungicide (Munkvold et al., 2001; Wise and Mueller, 2011), and hybrid seed disease resistance (Munkvold et al., 2001; Mueller and Pope, 2009; Paul et al., 2011).

Dry matter intake and energy concentration of corn silage determine the potential energy intake and, therefore, the animal’s performance (Allen et al., 2003). In an analysis of 20 experiments, increasing the NDF content of corn silage fed to dairy cattle linearly resulted in lower milk yield (Briceno et al., 1987). Digestibility of NDF is a function of the potentially digestible fraction, the rate of digestion, and the passage rate (Allen and Mertens, 1988). An increase of 1 percentage unit of NDF digestibility in vitro or in situ resulted in a 0.25-kg increase in FCM (Oba and Allen, 1999). Greater lignification of plant cell walls may increase bulk density of the feedstuff or require greater energy concentration of the diet to meet the nutritional requirements (Allen et al., 2003).

Haerr et al. (2015) reported a decreasing linear relationship between number of applications of fungicides and DMI, but constant milk production among treatments. Therefore, cows fed corn silage treated with foliar fungicide tended to have better feed conversion values than those fed untreated corn silage (Haerr et al., 2015). However, ideal time of fungicide application on corn to produce a high quality feedstuff remains to be determined. The objective of this study was to determine the effect of various foliar fungicide treatments on corn in relation to DMI, milk production, and milk composition when fed to Holstein cows.

MATERIALS AND METHODS

Corn

The corn hybrid planted was the Pioneer 1498 CHR RR + Pioneer 1498 RR refuge 2014 Variety (Johnston, IA), the purpose of which is silage. Reaching maturity in 114 d, this variety is marketed as drought tolerant, with high yields and digestibility. This hybrid is resistant to Gray Leaf Spot (caused by the disease *Cercospora zeae-maydis*), Northern Leaf Blight (caused by the fungus *Exserohilum turcicum)*, and Fusarium Ear Rot (caused by the fungus *Fusarium verticillioides).* Also, the hybrid contains a transgenic gene for suppression of corn earworm (*Helicoverpa zea*). Corn seeds were planted at a latitude 40°04’58.8”N and longitude 88°13’08.4”W on May 19, 2014 and treatments were randomly assigned to 1 of 4 0.8-ha plots. Treatments were as follows: corn receiving no foliar fungicide application **(CON)**; corn receiving one application of pyraclostrobin ($C_{19}H_{18}ClN_3O_4$) and fluxapyroxad ($C_{18}H_{12}F_5N_3O$)

(**PYR+FLUX**), foliar fungicide (Priaxor; BASF Corp.) at a rate of 0.15 kg/ha of active ingredient (a.i.)/ha at corn vegetative stage 5, where emergence of the fifth leaf is visible (**V5**; Mueller and Pope, 2009); corn receiving two applications of foliar fungicides, PYR+FLUX at 0.15 kg of a.i./ha at corn vegetative stage 5, and PYR+FLUX at 0.15 kg of a.i./ha at corn vegetative stage 8, where the emergence of eighth leaf is visible (**V5/V8**; Mueller and Pope, 2009); corn receiving three applications of foliar fungicides, PYR at 0.15 kg of a.i./ha at corn vegetative stage 5, PYR at 0.15 kg of a.i./ha at corn vegetative stage 8, and a mixture of pyraclostrobin ($C_{19}H_{18}ClN_3O_4$) + metconazole ($C_{17}H_{22}ClN_3O$) foliar fungicide (**MET**; Headline AMP®; BASF Corp.) at 0.15 kg of a.i./ha at corn reproductive stage 1, when the silks are fully extended (**V5/V8/R1**; Mueller and Pope, 2009).

The fungicide application dates were June 26, July 11, and July 23, 2014. Applications of foliar fungicide were applied with a 4430 Case IH ground sprayer (CNH Industrial, London, UK) at 482 kPa of pressure with a 73-60-110 10 VS nozzle tip spraying at a volume of 168.54 L/ha. At each application, the sprayer was driven through all plots, even those not receiving fungicide, to equalize damage to the plants.

During the growth of the corn, foliar disease evaluation occurred four separate times. Evaluations occurred at vegetative stage 7 (V7; July 5, 2014), reproductive phase 1 (R1; July 21, 2014), reproductive phase 3 (R3; August 8, 2014) and reproductive phase 4 (R4; August 15, 2014). Ten plants within each treatment were randomly selected for disease evaluation at each time point. Disease severity, as a percentage of leaf area, was estimated using three leaves: the ear leaf, one leaf above the ear leaf, and one leaf below the ear leaf from each selected plant; a method validated by Reis et al. (2007). The same evaluator looked at the plants at each evaluation to minimize error. Data for mean environmental temperature for Champaign-Urbana, IL and total rainfall were collected from the time seeds were sown until harvest (Illinois State Water Survey, Prairie Research Institute, Champaign, IL).

One week prior to harvest, 18 plants from each treatment (n = 72) were cut, collected, and removed from the field. Chopped directly above the first node in relation to the soil, the length of each corn stalk was measured. Furthermore, the number of green leaves and yellow leaves on the corn were counted. Corn ear weight was also measured for each plant.

Upon corn reaching ~32% DM, harvest for CON and V5 occurred on September 2, 2014; and for V5/V8 and V5/V8/R1 on September 3, 2014. Corn was chopped and processed using a New Holland FP240 forage chopper (CNH Industrial, London, United Kingdom). The processor was set to a 1.9-cm theoretical length of chop and a kernel processor was used to improve digestibility of the silage. At the time of harvest, a minimum of three samples of chopped corn material from each treatment was composited to estimate the dry matter. The DM for CON, V5, V5/V8, and V5/V8/R1 measured 31.1%, 33.3%, 30.2%, and 31.7%, respectively. Chopped corn was transported by H&P forage wagons (H & S Manufacturing Company Inc., Marshfield, WI) from the field to scale (Mettler Toledo, Columbus, OH) where the weight of the wagon was recorded. Once at the storage site, chopped corn material was ensiled in 2.74-m diameter bags using an AG bagger (Ag Bag Systems, St. Nazianz, WI). The calculated dry matter of the silage from each treatment allowed for individual adjustments to the bagger, preserving each treatment in a uniform manner. Additionally, an inoculant (Silo King, Agri-King, Fulton, IL) was added at a rate of 115 g / 1000 kg of corn to better preserve the corn silage. Corn silage was ensiled for at least 245 d before opening. The trial finished 336 d post ensiling.

Animals

University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee approved all experimental protocols. Sixty-four Holsteins 2.2 ± 0.8 parity, 134 ± 37.6 DIM, and 626 ±77 kg of BW were randomly selected and assigned to one of four treatments in a completely randomized block design. Distributed among 16 blocks, cows were assembled in groups of four using lactation number, previous lactation 305-d production, DIM, and BCS as variables to limit their influence on the outcome of the study. One week prior to the start of the experiment, a covariate period (week -1), measured the baselines of desired variables. A generic corn silage, not used in a treatment, was fed to all cows during the covariate period. Allotting time for adjustments to the treatment over the next 4 wk (week 1 to week 4), only data collected during wk 5 were used to make inferences regarding the treatments.

All cows were fed a mid-lactation diet (Table 2.1) supplying 100% of the NRC (2001) requirements for energy and all nutrients. Treatments only differed in the application of foliar fungicide on corn subsequently ensiled for measuring fungicide effects during lactation. All cows were fed 45% of dietary DM as corn silage.

All cows were fed once daily at 1500 h and housed in tie stalls, meeting or exceeding space requirements specified in the AG Guide (FASS, 2010). Furthermore, cows had feed and water available at all times of the day. Split between two barns (9.8 m apart), all treatments were placed in each barn to reduce the possibility of a barn effect. Barn 1 was milked daily at 4 h and 16 h, and barn 2 was milked daily at 6 h and 18 h. Temperature and humidity were monitored in 5-min intervals for barn 2 by using HOBO Pro Logger (Onset Computer Corp., Pocasset, MA). Temperature-humidity index (THI) was calculated using the following equation: THI= 0.8 × air temperature (°C) + [relative humidity × (air temperature (°C) – 14.4)] + 46.4 (McDowell et al., 1976). Over the duration of the trial, the average THI measured was 71.3 ± 6.0.

Sample Collection

Feed ingredients and TMR samples were obtained weekly and dried in a forced air oven at 110°C for 24 h (AOAC International, 1995a) to analyze DM content. Using the DM from each ingredient, the diet was updated weekly to ensure constant diet composition. Dietary DM of corn silage was sampled twice weekly to account for more frequent changes in its composition. Also using dietary DM, updates to inclusion of corn silage in the diet occurred twice a week. Weekly TMR samples and biweekly corn silage samples from each treatment were collected and stored at -20°C for later nutrient analysis. Samples of treatment TMR and corn silage were separated weekly using the Penn State Particle Separator (Nasco, Fort Atkinson, WI.) to analyze the particle size distribution of diets (Kononoff et al., 2003).

Once all samples were collected, weekly samples of TMR (wk 1 to wk 5) and biweekly samples of corn silage (wk 1 to wk 5) were composited into five samples per treatment of TMR and five samples per treatment of corn silage for laboratory analysis. All samples were analyzed for dietary DM, CP, soluble protein, NDF, ADF, fat, lignin, starch, and ash using a wet chemistry at a commercial laboratory (Dairy One, 2015). Using the NRC (2001), equations for TDN and NE_L were calculated.

Briefly, corn silage samples were dried in a forced air oven at 60°C (Goering and Van Soest, 1970). For analysis of ADF concentration, 0.5g-samples were individually and digested for 75 min as a group of 24 in 2 L of ADF solution in an ANKOM A200 digestion unit (Macedon, New York). Samples were rinsed 3 times with boiling water for 5 min in filtered bags and then soaked for 3 min in acetone, followed by drying at 105°C for 2 h (AOAC International, 2000; ANKOM, 2011). For analysis of lignin concentration, samples were initially subjected to ADF analysis, and residue digested as a group of 24 with 72% w/w sulfuric acid for 3 h in

ANKOM Daisy incubator (AOAC International, 2000; ANKOM, 2011). For analysis of NDF concentration, 0.5g-samples were weighted and digested for 75 min as a group of 24 in 2 L of NDF solution in ANKOM A200 digestion unit. Four milliliter of alpha amylase and 20g of sodium sulfate were added at the start of digestion. Samples were rinsed 3 times with boiling water for 5 min. After rinses, bags are soaked for 3 min in acetone, followed by drying at 105°C for 2 h (Van Soest et al., 1991; ANKOM, 2011).

Additionally, corn silage samples were evaluated for water-soluble carbohydrates, kernel processing score, and fermentation products (pH, lactic acid, acetic acid, propionic acid, ammonia, butyric acid, iso-butyric acid, total acid, NDF digestibility at 30-h, in vivo total digestibility at 30-h, and rate of digestion/hour) at the same commercial laboratory (Dairy One, 2015).

Briefly, volatile fatty acid analysis required weighted 50g-samples of corn silage to be blended at 20000 rpm for 2 min in 750 mL deionized water. Samples were filtered through cheesecloth, and filtered again with a disposable syringe filter. Acetic, propionic, butyric, and iso-butyric acid were analyzed using gas chromatography and 100ppm trimethylacetic acid with a Perkin Elmer Autosystem XL Gas Chromatograph. Lactic acid for corn silage samples was analyzed using YSI 2700 SELECT Biochemistry analyzer with a L-Lactate membrane. Digestibility of NDF was determined by incubating dry, ground samples in a buffer/rumen fluid mixture as described by Goering and Van Soest (1970) for 30-hr, under anaerobic conditions at 39°C. Lastly, a corn processing score and expected milk yields (milk lbs/ ton DM) were estimated (Dairy One, 2015). Corn processing score, also referred to as kernel processing score, is calculated by subtracting the percentage of starch that did not pass through the 4.75-mm sieve from the total percentage of starch (Ferreira and Mertens, 2005).

Additionally, corn silage was analyzed for mycotoxins which included, aflatoxin B1, aflatoxin B2, aflatoxin B3, aflatoxin G1, aflatoxin G2, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, vomitoxin, Trichothecene (T-2) and zearalenone at a commercial lab (Dairy One, 2015). Aflatoxin B1, aflatoxin B2, aflatoxin B3, aflatoxin G1, and aflatoxin G2 was determined using AOAC 994.08 (AOAC International, 2005). 3- acetyldeoxynivalenol, 15-acetyl DON and vomitoxin were determined using analytical procedures described by Trucksess et al. (1997) and MacDonald et al. (2005a). Trichothecene mycotoxin was determined using an analytical procedure as described by Croteau et al. (1994). Zearalenone was determined using an analytical procedure as described by MacDonald et al. (2005b). In brief, the mycotoxin sample is extracted from the corn using an acetonitrile/water (80/20) extraction method. Extracted mycotoxins samples are then prepared as solid phase extracts using a Triology MT3000 clean up column (Trilogy Analytical Laboratory, Washington, Missouri) and analyzed using a liquid chromatography-mass spectrometry technique.

Individual cow milk weights were recorded daily. Milk samples were collected at both milkings on d 2 and 4 of wk 5. Individual samples were composited using the proportion of milk yield at each sampling and preserved (800 Broad Spectrum Microtabs II, D&F Control Systems Inc., San Ramon, CA). Composited milk samples were evaluated for percentage of fat, protein, lactose, somatic cell count, MUN, other solids, and total solids using AOAC procedures (AOAC, 1995b) at the commercial lab (Dairy One, 2015). Using the laboratory results for the milk composition of cows, ECM and FCM were calculated: ECM = (12.82 × fat, kg) + (7.13 x protein kg) + (0.323 × milk, kg); 3.5% FCM = (0.4255 × milk, kg) + (16.425 × fat, kg). Moreover, feed conversion for actual milk kg/DMI, ECM/DMI, and FCM/DMI were calculated.

Evaluations of fecal score (**FS**) and general appearance (**GA**) for all cows occurred weekly. Fecal scores were evaluated on a 1 to 4 scale as follows: 1= liquid consistency, spreads in all directions once hits surface; 2 = a loose pile that holds some form when hits surface, but spreads; 3 = soft, and firm but not solid piles, slightly splatters upon impact; 4 = firm, dry, hard piles, does not spread upon impact. Cows with a FS ≤ 2 were considered to have digestive problems, whereas cows with FS > 2 were considered healthy. Additionally, GA was evaluated on a 1 to 3 scale as follows: 1 = bright, alert; 2 = depressed, temperature affected; 3 = reluctant to rise. Cows with GA ≥ 2 were classified as sick (altered), whereas cows with cows with GA < 2 were considered healthy.

Body weight and BCS were recorded at wk 5 for all treatments. Body condition score was assigned using a 1 to 5 scale, measuring in quarter increments (Ferguson et al., 1994). Three individuals evaluated BCS and the median value was used in analysis. Lameness scores (**LS**) were recorded for all treatments. Visual scoring of locomotion was assessed using a 1 to 5 scale; 1= normal; 2 = mildly lame, slightly irregular gait; 3 = moderately lame, favoring one or more limbs; 4 = severely lame; 5 = extremely lame (Bicalho et al., 2007). Cows were considered lame if the lameness score was ≥ 2.

Blood was collected from the tail vein or artery 1.5 h post feeding on d 4 of wk -1 and d 4 of wk 5 for all cows. Blood samples were centrifuged (959 × g for 15 min at 4°C). Serum and plasma samples were stored at -80°C within 2 h of blood collection. Once all samples were collected, serum samples from wk -1 and wk 5 were analyzed using commercially available kits for blood urea nitrogen (**BUN**), non-esterified fatty acids (**NEFA**), and glucose. Urea N was measured using a QuantiChrom Urea Assay kit (BioAssay Systems, Hayward, CA). Glucose was measured using a glucose auto kit (Wako Diagnostics, Richmond, VA). Non-esterfied fatty acids were measured using a non-esterified fatty acid auto kit (Wako Diagnotics, Richmond, VA.)

Density of corn silage bags was calculated biweekly for all treatments. A forage probe (Dairy One Forage, Ithaca, NY) was attached to a drill and drilled into five spots on the face of the corn silage bag (upper left, bottom left, bottom right, upper right, and center) to estimate the length of fresh samples in centimeters. Fresh samples were weighed and then dried to obtain DM, both used to calculate the as fed and dry matter density (kg/m^3).

Temperature data loggers (Maxim Integrated, San Jose, CA.) measured corn silage aerobic stability. Loggers were set to record 5-min intervals for 48 h. Treatment corn silage was collected before feeding from 5 places on the corn silage bag face. Representative samples of 2.3 kg of corn silage were aerated and placed into a 19L-bucket (Blain's Farm and Fleet Supply Inc., Urbana, IL.) with 3 buckets per treatment. Three temperature loggers were placed in each bucket and all buckets were kept in ambient temperature for the 48-h of data collection. Three temperatures loggers were placed in a bucket with no corn silage to measure the environmental temperature. Corn silage aerobic stability was repeated four times throughout the experiment.

Cow activity was monitored using HOBO pendant G Loggers (Hobo Pendant G Acceleration Data Logger, Onset Computer Corp.). Each logger was laterally attached to the left hind leg using vet wrap (3M; Indianapolis, IN). Loggers were attached to randomly selected cows in all treatments, in both barns. Set to record at 60-sec intervals, loggers measured the cows standing and lying behavior validated by Ledgerwood et al. (2010). Data from loggers were collected every 2 wk and reset on the same day for another 2 wk of attachment. Lying time, standing time, number of standing bouts, number of lying bouts, standing duration, and lying duration were calculated.

Statistical Analysis

Data collected during wk 5 were used for treatment inferences about the desired variables including DMI, milk production, milk components (protein, lactose, and urea N), serum metabolites (glucose, NEFA, and urea N), feed conversion calculations, and behavior. Data from wk 5 were reduced to means prior to statistical analysis. Statistical analysis was performed using SAS (v. 9.4, SAS Institute Inc., Cary, NC). Mixed models were created using the MIXED procedure to analyze wk 5 data using the fixed effects of treatment, block, and covariate. For DMI, milk yield, BW, BCS, glucose, NEFA, and BUN, wk -1 was used as the covariate. In the model, cow was the experimental unit and considered as a random effect. Behavioral data were categorized into standing duration, standing time, standing bouts, lying duration, lying time, and lying bouts using SAS. Using a mixed model, parameters were analyzed with treatment as a fixed effect and cow as a random effect. Three contrasts were used. Contrast 1: CON vs. TRT compared control to the average of treatments fed corn silage sprayed with foliar fungicide (V5, V5/V8, and V5/V8/R1); contrast 2: V5 vs. V5/V8 compared the treatment fed corn silage sprayed at V5 to the treatment fed corn silage sprayed at V5 and V8; and contrast 3: V5/V8 vs. V5/V8/R1 compared the treatment fed corn silage sprayed at V5 and V8 to the treatment fed corn silage sprayed at V5, V8, and R1. The degree of freedom method was Kenward-Rogers (Littell et al., 1998). General appearance scores, lameness scores, and fecal scores were analyzed as a binomial distribution (sick or healthy) using the GLIMMIX procedure in SAS. Corn silage, TMR quality, and density descriptive variables are presented as mean ± SD values. Aerobic stability for CON, V5, V5/V8, V5/V8/R1, and room temperature were calculated using the GLM procedure in SAS.

The distribution of residuals were evaluated for normality and homoscedasticity. Extreme outliers were removed for BCS (n = 1), milk fat (n = 2), ECM (n = 2), FCM (n = 2) and feed conversion (n = 2). Somatic cell count and BUN were log transformed for better distribution of values and variance of residuals. The data were back transformed and presented as the least squares means values for SCC and BUN. Statistical significance was declared at *P*-value less than or equal to 0.05 and a tendency was declared at *P*-value less than or equal to 0.10.

RESULTS

Yield of total corn mass in CON, V5, V5/V8, and V5/V8/R1 totaled 75.7, 76.1, 76.9, and 77.6 x 10^3 kg/ha, respectively. Signs of foliar disease from in field evaluations were not present at the first two evaluation dates, either because no was disease present or it was not detectable by the evaluator. On the third evaluation of foliar disease, at R3, corn plants in CON had an average of 2.5 % of leaf area infected with Gray Leaf Spot, and 1% of leaf area infected with common rust; for corn plants in V5 an average of 1% of leaf area was infected with Gray Leaf Spot; for corn plants in V5/V8 an average of 1% of leaf area was infected with common rust, and for plants in V5/V8/R1 no disease was found. On the fourth evaluation of foliar disease at R4, corn plants in CON had an average of 6% of leaf area infected with Northern Leaf Blight, 1% of leaf area detected with common rust; corn plants in V5 had an average of 3.5% of leaf area infected with Northern Leaf Blight, an average of 1% of leaf area infected with common rust V5, and an average of 1.3% of leaf area infected with Gray Leaf Spot. No signs of foliar disease were found in V5/V8 and V5/V8/R1 at the fourth evaluation. Total rainfall during the corn growing season was 53 cm. Average temperature during the corn growing season was 22.2 ± 5°C.

From the collection of corn prior to harvest, mean length of the corn stalks measured 339.3 ± 9.2, 328.11 ± 13.5, 329.2 ± 7.2, and 333.9 ± 11.4 cm for CON, V5, V5/V8, and V5/V8/R1, respectively. Number of green leaves on the corn plant averaged 11.06 ± 0.73, 11.56

± 0.62, 11.06 ± 0.80, 10.95 ± 1.00 for CON, V5, V5/V8, and V5/V8/R1, respectively. Number of yellow leaves for CON, V5, V5/V8, and V5/V8/R1 averaged 1.61 ± 0.78, 0.72 ± 0.46, 1.22 ± 0.73, and 1.20 ± 0.62, respectively. Collected corn ears averaged 336 ± 42, 365 ± 34, 349 ± 33, and 388 ± 37 g for CON, V5, V5/V8, and V5/V8/R1, respectively.

Corn Silage Quality

Analyzed nutrients and fermentation products from corn silage sampled biweekly during the experimental period are in Table 2.2. The mean dry matter densities of the treated corn silages were 232 ± 87, 241 ± 80, 246 ± 74, and 231 ± 85 kg/m^3 for CON, V5, V5/V8, and V5/V8/R1, respectively. The mean kernel processing scores of composited corn silage samples collected during the experimental period were 60.0 ± 1.6, 64.2 ± 2.2, 64.1 ± 3.9, and 62.7 ± 2.4 for CON, V5, V5/V8, and V5/V8/R1, respectively. Corn silage and room temperatures measured over 48-h averaged 32.9, 33.7, 31.7, 34.1, and 24.12 (SE = 0.10) for CON, V5, V5/V8, V5/V8/R1, and room temperature, respectively. The particle size distribution for corn silage in CON, V5, V5/V8, and V5/V8/R1 using the Penn State Separator for the 19-mm pore size was 19.9 ± 0.08, 21.6 ± 0.09, 24.2 ± 0.15, and 21.9 ± 0.09%; for the 8-mm pore size 58.8 ± 0.10, 58.5 ± 0.10, 57.5 ± 0.10, 59.7 ± 0.12%; for pore size 1.2 mm 18.7 ± 0.04, 18.5 ± 0.03, 17.1 ± 0.01, and 17.1 ± 0.02%; and for the pan 2.8 ± 0.1, 0.9 ± 0.0, 1.2 ± 0.01, and 0.6 ± 0.00%, respectively. Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, 3- acetyldeoxynivalenol, 15-acetyldeoxynivalenol, T-2, and zearalenone toxins were not detected in CON, V5, V5/V8, or V5/V8/R1 silages. Mean concentrations of vomitoxin in CON, V5, V5/V8, and V5/V8/R1 silages were 0.25 ± 0.35, 0.60 ± 0.00, 0.65 ± 0.07, 0.60 ± 0.00 ppm, respectively.

Nutrient Composition of Basal Diet

The analysis of nutrients for the TMR during the experimental period is in Table 2.3. The TMR particle distribution for CON, V5, V5/V8, and V5/V8/R1, for the 19-mm pore size were 9.9 ± 0.02, 11.0 ± 0.08, 12.9 ± 0.09, 13.6±0.08%; for the 8-mm pore size 42.5 ± 0.07, 40.6 ± 0.05, 40.6 ± 0.08, 38.0 ± 0.08%; for pore size 1.2 mm 37.5 ± 0.08, 37.1 ± 0.04, 37.5 ± 0.07, 35.9 ± 0.05%; and for the pan 10.1 ± 0.03, 11.6 ± 0.03, 8.9 ± 0.05, and 13.0 ± 0.07%, respectively.

Health, Fecal scores, and Activity

The GA values were 0.04, 0.14, 0.17, and 0.12 for cows in CON, V5, V5/V8, and V5/V8/R1, respectively. A treatment effect was not observed for GA when comparing cows in CON to cows fed treated corn silages (V5, V5/V8, V5/V8/R1; $P = 0.14$). Fecal score values for cows in CON, V5, V5/V8, and V5/V8/R1 were 0.19, 0.25, 0.33, and 0.23, respectively. No differences for fecal score were observed when comparing cows in CON to cows fed foliar fungicide treated corn silages (V5, V5/V8, and V5/V8/R1; $P = 0.48$). Lameness scores were 0.03, 0.11, 0.01, and 0.10 for CON, V5, V5/V8, and V5/V8/R1, respectively. A treatment effect was not observed for LS when comparing cows in CON to cows fed foliar fungicide treated corn silage (V5, V5/V8, and V5/V8/R1; $P = 0.11$). No differences in lying time were observed for cows in CON when comparing total time spent lying each day to the average time spent lying of cows in V5, V5/V8, and V5/V8/R1 (Table 2.4, $P = 0.74$).

Intake, BW, and BCS

Dry matter intake, BW, and BCS for each treatment are in Table 2.5. No treatment differences were observed for DMI ($P = 0.75$) and BCS ($P = 0.69$). Body weight of cows in V5/R1/R3 was lower than cows in V5/R1 ($P = 0.03$).

Milk Yield and Feed Conversion

Milk yield and feed conversion are in Table 2.5. Milk yield did not differ among treatments (*P* = 0.55). Cows in V5 tended to have higher 3.5% FCM than cows in V5/V8 (*P* = 0.07). Cows in V5 tended to have higher ECM than cows in V5/V8 (*P* = 0.07).

Milk Composition

Milk composition is in Table 2.5. Milk fat yield tended to be lower for cows in V5/V8 when compared with cows in V5 (*P* = 0.10). Milk lactose concentration tended to be higher for the average of treatments fed corn silage treated with fungicide when compared with CON (*P* = 0.09). Milk urea nitrogen concentration for cows in V5/V8/R1 was greater than cows in V5/V8 (*P* = 0.03).

Serum Metabolites (BUN, glucose, NEFA)

Serum metabolite data are in Table 2.5. No differences were observed in serum BUN concentrations for cows in CON than the average of the corn silage treated with fungicide (*P* = 0.35). Serum glucose concentrations for cows in V5/V8/R1 were higher than cows in V5/V8 (*P* = 0.04). Serum NEFA concentrations for cows in CON were lower than the average of treatments fed corn silage treated with fungicide (*P* = 0.05).

DISCUSSION

The aim of this study was to determine the efficacy of applying foliar fungicide on corn destined for corn silage in enhancing corn silage quality and, as a result, improve DMI, milk production, and milk composition when fed to Holstein cows.

In the present study, we observed no differences in DMI of mid lactation cows fed corn silage from corn treated with foliar fungicide compared with cows fed control corn silage (Table 2.5). Because cows used in this study were post peak lactation, we were not expecting an increase in DMI. The results of others confirm our findings. Kertz et al. (1991) evaluated 18 experiments over a 6-yr period and reported cows' DMI peaked around wk 10 but remained constant for the second 10 wk period. Contrary to our findings, Haerr et al. (2015) reported a trend for decreased DMI of mid lactation cows fed foliar fungicide treated corn silage when compared with control. Although, direct comparison of DMI of cows in the current study and Haerr et al. (2015) is confounded with different inclusion rates of forages and corn silage in the diet. Total forage DM in the current study was 57%, with corn silage comprising 45% of dietary DM. Haerr et al. (2015) included 48% of DM forage, with corn silage included at 35% of dietary DM. Additionally, variation in field and growing season for corn may have accounted for differences in experimental results. Body weight of cows in V5/V8/R1 was significantly lower than cows in V5/V8. Yet, DMI as a percentage of BW was not different for cows in V5/V8/R1 compared with cows in V5/V8. Bal et al. (1997) reported DMI as a percentage of BW for cows, 75 DIM, fed one of two different corn silages either harvested at the 2/3 maturity line or black line to be 3.77% and 3.79%, respectively. Our data for DMI as a percentage of BW are similar to the results of Bal et al. (1997). Therefore, we do not think differences of body weight to be physiologically relevant. Furthermore, energy balance is better indicated by BCS compared to BW (Kertz et al., 1991), which was not statistically different when comparing cows in V5/V8 to cows in V5/V8/R1.

Milk yield was unchanged for cows fed foliar fungicide treated corn silage compared with cows fed control corn silage (Table 2.5). Cows in V5 tended to produce 2.11 kg/d more milk than cows in V5/V8. It may be that earlier applications of foliar fungicide on corn results in a higher quality feedstuff. Blandino et al. (2012) reported that applications of fungicide on corn at the mid-stem elongation stage to significantly increase grain yield when compared with control. Cows fed corn silage treated one time with foliar fungicide also had numerically greater milk yield when compared with cows fed corn silage with increasing frequencies of fungicide application (Haerr et al., 2015). Greater differences in milk yield may have been seen if the group in the current study were milked 3 times/d instead of 2 times/d. Cows were milked 3 times/day in Haerr et al. (2015) and yielded (34.9 kg/d) on average, 4.4 kg/d more milk when compared with cows milked 2 times/d (30.0 kg/d) in the present study. Amos et al. (1985) reported milking cows 3 times/d resulted in 18.5% higher milk yield than cows milked 2 times/d. Relatively lower milking yield in the present study may also have been the result of heat stress in the cows. Igono et al. (1992) determined critical values for THI to be a mean of 72, minimum of 64, and maximum of 76. Therefore, according to the THI and heat stress definition from McDowell et al. (1976), cows in the current study were on the lower threshold of experiencing heat stress (71.3 ± 6.0). McDowell et al. (1976) observed 17% higher total milk yield from first lactation cows in the winter compared to a separate group in the summer months. The calculated temperature humidity index of the current study was 7.7 units above the calculated temperature humidity index of Haerr et al. (2015), and may help further explain lower milk yields in the current study. Had the trial been conducted in the fall or earlier in the season, milk yield may have been different with the absence of heat stress.

Cows in V5 tended to yield 0.16 kg/d more milk fat when compared with cows in V5/V8 (Table 2.5). These results are similar to those of Haerr et al. (2015), where cows fed corn silage treated once with foliar fungicide, yielded 0.12 kg/d more milk fat when compared with cows fed corn silage from corn treated twice with foliar fungicide. In the current study, milk fat concentration was not different for cows in V5 when compared with cows in V5/V8. Therefore, greater yield in milk fat for cows in V5 is related to increased milk production when compared with cows in V5/V8. Kendall et al. (2009) reported greater milk fat yield from early lactation cows fed anhydrous NH_3 treated wheat straw compared to control. In that study, the authors hypothesized greater milk fat yield to be the result of application of anhydrous NH_3 on wheat producing a more digestible fiber. Blandino et al. (2012) reported earlier applications of foliar fungicide on forage plants decreased disease symptoms more than applications later in development. Decreased diseased symptoms on corn plants may reduce the lignification within the cell wall and, therefore, reduce the concentration of fiber. The NDFD 30-h (Table 2.2) of corn silage in V5 and in V5/V8/R1 was, respectively, 3.8 and 2 units greater than the corn silage in V5/V8. Since digestibility in dairy cows is linked to both the passage rate of a forage and the portion that is digestible, applications of fungicide at V5 and V5/V8/R1 may reduce the concentration of fungal pathogen and reduce lignification in the corn stalk, resulting in a more digestible feedstuff. Further research is needed to separate the effects of increased application frequency on corn silage from of timing of application.

The tendency for greater milk fat yield of cows in V5 compared with cows in V5/V8 resulted in increased 3.5% FCM and ECM. Increases in 3.5% FCM and ECM yield may be the result of improved in NDFD of corn silage for applications of foliar fungicide at V5. In a meta-analysis, an increase of 0.25 kg of 4% FCM yield was associated with a 1-unit in NDF digestibility (Oba and Allen, 1999). More recently, a 1 percentage unit increase in forage in vitro NDFD increased 4% FCM by 0.18 kg/d (Kendall et al., 2009). Our data suggest that applications of fungicide at V5 may yield a more digestible corn silage, as indicated by increased milk fat yield, and, therefore, higher 3.5% FCM and ECM.

Kendall et al. (2009) observed small increases in the concentration of milk lactose for cows fed a highly digestible forage compared with cows fed a lowly digestible forage. The authors concluded small changes in milk lactose concentration could be attributed to differences in fiber digestibility; the diet containing a more digestible fiber producing greater milk lactose concentration. Furthermore, Oba and Allen (1999) analyzed seven studies with variation in where NDF digestibility either evaluated in situ or in vitro and reported increased milk lactose content for higher digestible NDF forages. Applications of foliar fungicide on corn may result in a more digestible feedstuff as indicated by an increase in milk lactose.

Management tools to assess the quality of silage include aerobic stability and density. Corn silage in V5/V8/R1 was the least stable over the 48-h with an increase of 3.7, 2.4, and 9.9°C over corn silage in CON, V5/V8, and room temperature, respectively. The decrease in aerobic stability of corn silage in V5/V8/R1 may be indicated by a decrease in lactic acid, an increase pH, and a decrease in water-soluble carbohydrates (Table 2.2). Microbes use the lactic acid and water-soluble carbohydrates as substrates for growth thus increasing the temperature of the silage. Growing and multiplying fungi change the chemical profile of silage, reducing the lactic acid, raising the pH, and thus decreasing the nutritional value (Pahlow et al., 2003). Density of packed silos also impact the aerobic deterioration and stability of the corn silage (Wilkinson and Davies, 2013). Lower silage density and, therefore, more porosity allows oxygen to permeate into silo (Wilkinson and Davies, 2013) directly influencing bacterial growth. Corn silage in V5/V8/R1 had a reduction of 15 kg/m^3 in DM density when compared to corn silage in V5/V8. Although the packing procedures for all treatments were similar, less packing of V5/V8/R1 may have allowed greater air into the bag and the start of aerobic deterioration of nutrients in V5/V8/R1. The lack of firmness in a silo could have contributed to the growth of undesirable bacteria such as enterobacteria, molds, or yeasts (Pahlow et al., 2003) therefore affecting the pH, aerobic stability, lactic acid, and nutrients composition of the silage (Cherney and Cherney, 2003).

Milk urea nitrogen concentration for cows in V5/V8/R1 increased 2.1 mg/dL compared to cows in V5/V8 (Table 2.5). The difference in MUN may be explained by a decrease in the concentration of sugar of corn silage in V5/V8/R1 compared to corn silage in V5/V8. Concentration of water soluble carbohydrates of corn silage in V5/V8/R1 was 0.6 percentage units of DM less than corn silage in V5/V8. Reducing the sugar content of a diet, and therefore the increasing the ratio of NDF:starch in the diet has shown to linearly increase the MUN concentration (Beckman and Weiss, 2005). Milk in V5/V8/R1 had increased concentration of MUN compared to V5/V8, possibly as a result of decreased aerobic stability and poor DM density causing differences in nutrient composition of corn silage in V5/V8/R1 compared to V5/V8.

In the present study, cows in V5/V8/R1 had increased serum concentrations of glucose compared to cows in V5/V8. Haerr et al. (2015) reported decreased concentration of serum glucose in cows fed foliar fungicide treated corn silage compared to control. In the current study, cows in V5 and in V5/V8 had numerically decreased concentrations of glucose compared to control. Although, cows in V5/V8/R1 did not follow a similar pattern, with an increased concentration of glucose compared to control. Increased blood concentration of cortisol, a direct result of physiological, nutritional, or psychological stress, increased the blood concentrations of glucose; as cortisol acts as an antagonist for insulin secretion and decreased glucose uptake by the peripheral tissues (Pechova and Pavlata, 2007). It is possible cows in V5/V8/R1 were more sensitive to small stresses resulting in decreased glucose uptake by the peripheral tissues.

CONCLUSIONS

Cows receiving corn silage treated with foliar fungicide had similar DMI and milk yield when compared with cows receiving control. Applications of foliar fungicide at V5 fed to cows tended to yield more milk fat, and greater 3.5% FCM and ECM than V5/V8. Cows fed corn silage treated with foliar fungicide had increased milk lactose concentration than CON. Applying fungicide at V5/V8 to the corn and ensiled as corn silage fed to dairy cattle does not seem to be as beneficial as applications of foliar fungicide at V5 and V5/V8/R1.

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TABLES AND FIGURES

Table 2.1. Ingredient composition of the lactation diet fed to cows throughout the experiment.

Ingredient	% of DM
Alfalfa hay	12.1
Corn silage[1]	44.95
Wet brewers grain	5.6
Dry ground corn grain	15.0
Soybean meal, 48% CP	6.6
Expeller soybean meal[2]	3.3
Soy hulls	7.1
Sodium bicarbonate	0.71
Limestone	0.12
Dicalcium phosphate	0.33
Molasses, sugar beet	2.20
Energy Booster 100[3]	1.59
Biotin[4]	0.32
Salt (plain)	0.30
Mineral and vitamin mix[5]	0.17

[1] All treatments fed at 45% corn silage DM.
[2] SoyPlus® (West Central, Ralston, IA).
[3] Energy Booster 100® (Milk Specialties Global of Paris, IL.); 98% total fatty acids and less than 2% unsaponifiable fat.
[4] 1g ROVIMIX® Biotin (DSM, Heerlen, the Netherlands) contains 20 mg of biotin.
[5] Mineral and vitamin mix was formulated to contain 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg/kg of Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg of Se, 2,200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D_3, and 7,700 IU/kg of vitamin E.

Table 2.2. Mean chemical composition and standard deviation of nutrient composition, fermentation profile, energy content, and kernel processing score of corn silage treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**), or three applications of foliar fungicide (**V5/V8/R1**).

Item	Treatment[1]				SD[2]
	CON	V5	V5/V8	V5/V8/R1	
Corn silage composition					
DM % as fed	31.98	31.92	31.14	31.84	1.51
CP,% of DM	8.14	8.36	8.40	8.58	0.40
NDF,% of DM	42.06	44.34	43.76	43.18	2.82
NDFD[3], 30h	51.80	53.00	49.20	51.20	4.60
ADF,% of DM	26.62	27.34	27.76	27.46	2.23
Fat,% of DM	3.70	3.80	3.68	3.66	0.22
Lignin,% of DM	3.30	3.78	3.36	3.34	0.71
Soluble CP,% of DM	55.80	51.60	55.40	56.40	1.82
Starch,% of DM	35.76	33.66	34.06	35.08	3.44
WSC[4],% of DM	2.22	2.42	2.52	1.92	0.45
Ash,% of DM	4.16	4.36	4.33	4.39	0.27
Ca,% of DM	0.24	0.23	0.22	0.22	0.02
P,% of DM	0.21	0.20	0.18	0.20	0.02
Mg,% of DM	0.13	0.12	0.12	0.12	0.01
K,% of DM	1.05	1.06	0.95	1.07	0.07
S,% of DM	0.12	0.12	0.11	0.11	0.01
Fe, ppm	108.80	103.80	103.20	90.40	15.42
Energy calculations[5]					
TDN, % of DM	72.70	72.00	70.60	71.60	2.51
NE_L, Mcal/kg	1.65	1.60	1.60	1.62	0.07
NE_G, Mcal/kg	1.06	1.01	1.01	1.04	0.08
NE_M, Mcal/kg	1.68	1.61	1.61	1.65	0.07
Fermentation products					
pH	3.96	4.04	4.00	4.04	0.23
Lactic acid, % of DM	4.59	3.93	3.82	3.07	1.27
Acetic acid, % of DM	1.01	1.04	1.11	1.84	1.08
Lactic/Acetic Ratio	4.56	4.16	3.87	2.62	2.17
Propionic Acid, % of DM	0.11	0.14	0.13	0.33	0.22
Ammonia N, % of DM	5.40	5.20	5.60	6.00	0.55
Kernel processing score[6]	59.98	64.16	64.12	62.28	3.86

[1] Treatment = Dietary treatments were CON (with no application of fungicide), V5, (with one application of fungicide at V5), V5/V8 (with two applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1). Average and standard deviation of five composited samples per treatment.
[2] Maximum within treatment SD.
[3] In vitro NDF digestibility, 30-h.
[4]Water soluble carbohydrates.
[5]NRC (2001).
[6]Total starch in sample - percentage of starch that did not pass through the 4.75 mm sieve.

Table 2.3. Mean chemical composition and standard deviation of diet fed to cows receiving corn silage treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**), or three applications of foliar fungicide (**V5/V8/R1**).

Item	Treatment[1]				SD[2]
	CON	V5	V5/V8	V5/V8/R1	
DM, %	45.28	47.18	45.00	45.86	2.40
CP, % of DM	16.22	15.88	14.74	15.34	0.76
ADF, % of DM	20.28	20.46	22.42	22.84	3.30
NDF, % of DM	31.60	32.42	34.94	35.36	3.64
Lignin, % of DM	2.66	3.18	3.54	3.72	0.80
Starch, % of DM	28.18	29.22	26.28	26.62	2.30
NDFD, 30h[3]	58.20	53.00	53.60	52.00	8.60
Crude fat, % of DM	5.02	5.12	5.02	5.30	0.34
Ash, % of DM	6.91	6.69	6.07	5.90	1.45
TDN, % of DM[4]	75.00	74.20	73.60	73.80	2.39
NE_L, Mcal/kg[4]	1.76	1.76	1.74	1.74	0.06
Ca, % of DM	1.01	0.89	0.79	0.68	0.52
P, % of DM	0.36	0.36	0.35	0.35	0.02
Mg, % of DM	0.22	0.21	0.22	0.21	0.02
K, % of DM	1.26	1.26	1.30	1.37	0.18
Na, % of DM	0.21	0.22	0.22	0.20	0.02
S, % of DM	0.23	0.23	0.21	0.22	0.02
Fe, ppm	446.80	400.00	383.00	346.80	211.99
Zn, ppm	89.60	91.60	86.20	81.80	8.26
Cu, ppm	17.60	17.00	16.20	15.60	2.30
Mn, ppm	91.00	84.40	80.00	74.60	23.53
Mo, ppm	1.20	1.16	1.02	1.04	0.22

[1]Treatment = Dietary treatments were CON (with no application of fungicide), V5, (with one application of fungicide at V5), V5/V8 (with 2 applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1). Average and standard deviation of five composited samples per treatment.

[2]Maximum within treatment SD.

[3]In vitro NDF digestibility, 30-h.

[4]NRC (2001).

Table 2.4. Least squares means and associated standard error for standing and lying behavior for cows fed corn silage treated with no foliar fungicide (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**) or three applications of foliar fungicide (**V5/V8/R1**).

	Treatment[1]					*P*-value		
						Contrasts[2]		
	CON	V5	V5/V8	V5/V8/R1	SEM	CON vs. TRT	V5 vs. V5/V8	V5/V8 vs. V5/V8/R1
Standing time, min	696.01	621.62	720.17	798.73	49.1	0.74	0.16	0.25
Standing duration, min	72.54	66.57	84.15	93.60	14.7	0.57	0.39	0.64
Standing bouts, n [3]	11.54	10.93	10.86	11.05	1.1	0.61	0.97	0.90
Lying time, min	743.99	818.38	719.83	641.27	49.6	0.74	0.16	0.25
Lying duration, min	56.81	60.60	57.02	51.11	4.7	0.91	0.58	0.36
Lying bouts, n [3]	15.54	16.90	14.72	14.36	1.9	0.92	0.40	0.89

[1] Treatment = Dietary treatments were CON (with no application of fungicide), V5, (with one application of fungicide at V5), V5/V8 (with two applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1).

[2] Contrasts were CON vs TRT = no fungicide application (CON) with that of the average of the three treatments with fungicide application; V5 vs. V5/V8= fungicide application at V5 compared with fungicide application at V5 and V8; V5/V8 vs. V5/V8/R1= fungicide application at V5 and V8 compared with fungicide application at V5, V8, and R1.

[3] n= Number of bouts/24 h.

Table 2.5 Least squares means and associated standard errors for DMI**,** milk parameters response, and serum metabolites of cows in (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**), or three applications of foliar fungicide (**V5/V8/R1**).

	Treatment[1]					*P*-value		
						Contrasts[2]		
	CON	**V5**	**V5/V8**	**V5/V8/R1**	**SEM**	**CON vs. TRT**	**V5 vs. V5/V8**	**V5/V8 vs. V5/V8/R1**
DMI, kg/d	19.50	19.57	20.86	20.38	1.00	0.48	0.37	0.73
BW, kg	626	634	627	613	4.76	0.75	0.34	0.03
DMI / BW, %	3.12	3.25	3.18	3.35	0.17	0.47	0.74	0.48
BCS	3.00	3.04	3.05	2.98	0.04	0.57	0.95	0.21
Milk yield								
Milk, kg/d	30.55	31.17	29.06	29.33	0.98	0.55	0.14	0.84
3.5% FCM, kg/d [3]	30.19	32.42	28.58	30.87	1.47	0.79	0.07	0.27
ECM, kg/d [4]	29.15	31.35	27.76	29.63	1.37	0.78	0.07	0.33
Milk composition								
Fat, %	3.66	3.75	3.65	3.77	0.18	0.78	0.68	0.63
Fat, kg/d	1.07	1.16	1.00	1.10	0.06	0.74	0.10	0.28
Protein, %	2.78	2.81	2.85	2.74	0.06	0.74	0.59	0.21
Protein, kg/d	0.82	0.89	0.81	0.81	0.04	0.74	0.14	0.87
Lactose, %	4.63	4.77	4.76	4.72	0.06	0.09	0.85	0.65
Lactose, kg/d	1.39	1.52	1.37	1.42	0.07	0.60	0.16	0.62
MUN, mg/dL	14.58	13.78	13.59	15.65	0.64	0.75	0.84	0.03
SCC[5]	255.88	102.81	262.06	253.91	82.43	0.71	0.20	0.73
3.5% FCM/DMI, kg/kg	1.49	1.68	1.56	1.50	0.10	0.44	0.43	0.65
ECM/DMI, kg/kg	1.43	1.62	1.51	1.44	0.09	0.42	0.43	0.55
Milk/DMI, kg/kg	1.57	1.63	1.52	1.44	0.10	0.74	0.45	0.57
Serum metabolites								
Urea N, mg/dL[5]	22.38	23.48	23.12	25.75	1.43	0.35	0.80	0.32
Glucose, mg/dL	54.63	51.70	50.18	55.94	1.90	0.34	0.56	0.04
NEFA, mEq/L	0.09	0.11	0.12	0.11	0.01	0.05	0.42	0.75

(Table 2.5 continued)

[1] Treatment = Dietary treatments were CON (with no application of fungicide), V5, (with one application of fungicide at V5), V5/V8 (with two applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1).

[2] Contrasts were CON vs TRT = no fungicide application (CON) with that of the average of the three treatments with fungicide application; V5 vs. V5/V8= fungicide application at V5 compared with fungicide application at V5 and V8; V5/V8 vs. V5/V8/R1= fungicide application at V5 and V8 compared with fungicide application at V5, V8, and R1.

[3] 3.5% FCM = [(0.4255 × milk yield) + (16.425 × milk fat yield)].

[4] ECM = [(12.82 × milk fat yield) + (7.13 × milk protein yield) + (0.323 × milk yield)].

[5] Log transformed data presented as back transformed units.

CHAPTER III

Applications of foliar fungicide on corn for corn silage for ruminants I. Pyraclostrobin effects on corn plant composition

ABSTRACT

The objective of this study was to determine the effect of foliar fungicide applied at various times during the growing season of corn on the chemical composition of corn leaves, corn ears, and corn stalks. Treatments were replicated once and assigned to 1 of 8 0.4-ha plots of corn, as follows: control (**CON**), corn receiving no foliar fungicide application; treatment 1 (**V5**), where corn received a mixture of pyraclotrobin and fluxapyroxad foliar fungicide (Priaxor, BASF Corp.) corn vegetative stage 5 (V5); treatment 2 (**V5+R1**), where corn received two applications of foliar fungicide, a mixture of pyraclotrobin and fluxapyroxad at V5 and a mixture of pyraclostrobin + metconazole foliar fungicide (Headline AMP; BASF Corp.) at corn reproductive stage 1 (R1); and treatment 3 (**R1**), in which corn received one application of pyraclostrobin + metconazole foliar fungicide at R1. Evaluators at R1 and corn reproductive phase 3 (R3) reported signs of Gray Leaf Spot and Northern Leaf Blight on the foliage. Twenty-four individual corn plants from each treatment were collected R1 and R3 for weight and length measurement. At each collection, corn was disassembled into leaves, stalks, flag leaf, and ears for chemical analysis. At R3, corn in V5+R1 and R1 had less yellow leaves than CON and V5 (1.7, 1.5, 0.83 and 0.88 for CON, V5, V5+R1 and R1, respectively; P = 0.03), and treated corn were taller than untreated (2.5, 2.9, 3.1, and 2.8 m for CON, V5, V5+R1, and R1, respectively; P = 0.02). Fungicide treated corn stalks had greater lignin content (56, 64, and 50 g/kg DM for V5, V5+R1, and R1, respectively) compared with untreated (46 g/kg DM; P = 0.03), with the

greatest concentration from corn stalks in V5+R1. Fungicide treated corn leaves had less ADF content (331, 283, and 330 g/kg DM for V5, V5+R1, and R1; $P = 0.01$) and NDF content (584, 524, and 554 g/kg DM for V5, V5+R1, and R1; $P = 0.02$) compared with untreated leaves (333 g/kg DM ADF; 569 g/kg DM NDF, respectively). In conclusion, results suggest that applications of foliar fungicide at V5 and R1 together may have a synergistic interaction on the fibrous content within the corn leaves, producing a higher quality feedstuff for ruminants when fungal disease is present.

Keywords: Corn, foliage, fungicide ruminant nutrition

INTRODUCTION

Corn products are fed to a variety of livestock, including: dairy cows, beef cattle, poultry, and swine. In ruminant diets, corn silage is one of the most popular forages fed in the United States, with 14% of total corn production in 2014 devoted to its production (USDA, 2014). On a DM basis, whole plant corn silage composition is about 57% corn ears, 13% corn leaves, and 31% corn stalks (Kuehn et al., 1999). For beef feedlot diets, most nutritionists include corn silage in the diet at 14% of finishing diet (Klopfenstein et al., 2013). For dairy cow diets, corn silage represents 40 to 60% of the total mix ration in lactating diets. Dairy and beef producers favor inclusion of corn silage in the diet for its heterogeneous composition as a grain and a roughage, favorable palatability, and higher consistent quality when compared with other forages. Ruminant diets are physically limited on the amount of forage included in the diet due to its fibrous quality. Silages with greater NDF, ADF, and lignin content; as well as, lower NDF digestibility prevent producers from greater inclusion sin the diet.

Fungi can have a mutual relationship with corn plants, as they decompose organic matter, thus providing necessary nitrogen in the soil. But, fungi can also have a parasitic relationship with corn plants. Under certain weather conditions, fungal pathogens on the growing plant complete the last side of the disease triangle between host, pathogen, and environment. Both physical barriers such as cell walls, and chemical releases such as secondary metabolites aid plants in protecting from pathogens (Malinovsky et al., 2014). If fungi remain undetected on the plant surface, enzymes degrade cell walls and once inside produce toxins killing the plant tissue, thus providing nutrients for fungal growth (Sexton and Howlett, 2006). Plants have adapted to increasing the lignin concentration in the secondary cell wall, thus creating a tougher barrier for digesting when wounded or infected with a fungal pathogen or insect (Santiago et al., 2013).

Many reports have captured the impact of fungal pathogens on final corn yields. In 2013, 7.5% of the total estimated corn yield was lost to disease; more specifically, 12.2 million metric tons of a total yield of 330 million metric tons lost to foliar diseases on corn (Mueller and Wise, 2014). Under ideal weather and environmental conditions, a 1% unit increase in disease severity of Gray Leaf Spot, a foliar fungus caused by *Cercospora zeae-maydis,* caused a 47.6 kg/ha reduction in corn yield of a generic hybrid and a 35.7 kg/ha reduction in corn yield of a moderately tolerant hybrid (Nutter and Jenco, 1992; Ward et al., 1999). Furthermore, in a meta-analysis of 20 studies, a 10% unit increase in common rust on sweet corn resulted in an estimated 2.4 to 7.0% loss in yield (Shah and Dillard, 2006). Corn inoculated with Northern Leaf Blight (*Exserohilum turcicum* Pass.; teleomorph: *Setosphaeria turcicum)* suffered significant yield losses when compared with uninoculated corn (Wang et al., 2010). Less research is available on how fungal pathogens affect the nutritive quality of the leaves, stalk, and ear, individually. Inoculation of Northern Leaf Blight on corn resulted in a 52.6 g/kg of DM and a 41.2 g/kg of DM increase in NDF and ADF, respectively, when compared with the control corn plant (Wang et al., 2010).

In diseased fields, applications of foliar fungicides on corn can improve yields. A meta-analysis concluded that corn treated with pyraclostrobin fungicide applications increased the mean yield of corn 256 kg/ha (Paul et al., 2011). In a year with high incidence of common rust, fungicide application at vegetative stage six (V6), when six leaf collars are visible on the growing plant (Mueller and Pope, 2009), increased corn grain yield by 362.9 kg/ha compared with application at pre-tassel, when 6% of the total leaf area was diseased (Wright et al., 2014). Within the same study but a different year with low disease incidence, fungicide application on corn at V6 did not increase corn grain yields when compared to application pre-tassel (Wright et al., 2014).

Few studies have been conducted on how foliar fungicide applications on diseased corn affect the nutritive quality of various parts of the plant. The objective of this study was to determine the effect of foliar fungicide applied at various times during the growing season of the corn plant on the chemical composition of corn leaves, corn ears, and corn stalks.

MATERIALS AND METHODS

Field preparation

Before winter 2014, manure was applied to the field where corn would be planted in the spring of 2015. Land was tilled conservatively using a Case IH Tiger Mate II (CNH Industrial, London, UK), making just one pass. Seven soil samples were collected from various places in the field and sent to a commercial laboratory (Rock River Lab, Watertown, WI.) for soil analysis. Soil samples were analyzed for pH, buffer pH, organic matter, phosphorus, potassium, calcium, magnesium, boron, manganese, zinc, and cation exchange capacity (CEC). Data for mean environmental temperature for Champaign-Urbana, IL and total rainfall were collected daily from planting until harvest from the state climatologist office for Illinois (Illinois State Water Survey, Prairie Research Institute, Champaign, IL).

Corn

The corn hybrid was Pioneer 1417AMXRR 2015 variety (Johnston, IA), the purpose of which is silage. Comparative relative maturity (CRM) for this hybrid is reached at 114 d. The hybrid of corn is marketed for having an outstanding silage yield, whole plant digestibility, and silage crude protein values. The variety is resistant to Gray Leaf Spot (caused by the disease *Cercospora zeae-maydis*) and Northern Leaf Blight (caused by the fungus *Exserohilum* *turcicum).* Corn seeds were planted on 30 April 2015 using a John Deere 7200 tractor (Moline, IL.). Eight 0.4-ha plots of corn were planted (40°04'58.8"N 88°13'08.4"W) at a density of 16000 corn plants/ha.

Foliar fungicide application

Treatments were replicated once and assigned to 1 of 8 0.4-ha plots of corn. Treatments were as follows: control (**CON**), corn receiving no foliar fungicide application; treatment 1 **(V5**), where corn received a mixture of pyraclotrobin ($C_{19}H_{18}ClN_3O_4$) and fluxapyroxad ($C_{18}H_{12}F_5N_3O$) (**PYR+FLUX**), foliar fungicide (Priaxor, BASF Corp.) at a rate of 0.15 kg of active ingredient (a.i.)/ha at corn vegetative stage 5 (V5) where the emergence of the fifth leaf is visible (Mueller and Pope, 2009); treatment 2 (**V5+R1**), where corn received two applications of foliar fungicide, a mixture of PYR+FLUX at 0.15 kg of a.i./ha at corn vegetative stage five, and a mixture of pyraclostrobin ($C_{19}H_{18}ClN_3O_4$) + metconazole ($C_{17}H_{22}ClN_3O$) foliar fungicide (**PYR+MET**; Headline AMP; BASF Corp.) at 0.15 kg of ai/ha at corn reproductive stage 1 (R1) or when the silks are fully extended (Mueller and Pope, 2009); and treatment 3 (**R1**), in which corn received one applications of PYR+MET foliar fungicide at 0.15 kg of a.i./ha at corn reproductive stage 1.

Fungicide applications dates were 3 June 2015 (34 d post planting; corn stage V5), and 13 July 2015 (75 d post planting; corn stage R1). Applications of foliar fungicide were applied with a 4430 Case IH ground sprayer (CNH Industrial, London, UK) at 482 kPa of pressure with a 73-60-110 10 VS nozzle tip spraying at a volume of 168.54 L/ha. At each application, the sprayer was driven through all the treatments, even those not receiving fungicide to account for equal damage to the plant.

Disease evaluation

Two times during the growing season corn was evaluated for foliar disease. Evaluations occurred on 11 July 2015, at corn reproductive stage 1 (R1) and on 13 August 2015, at reproductive phase 3 (R3) when kernels are yellow, with a milk white fluid (Mueller and Pope, 2009). Ten plants within each treatment were randomly selected for evaluation at each time point. Disease severity, as a percentage of leaf area, was estimated using three leaves: the ear leaf, one leaf above the ear leaf, and one leaf below the ear leaf; a method validated by Reis et al. (2007). The same evaluator walked through the treatments in the field and evaluated the plants at both time points to minimize possible error.

Crop collection

Corn stalks, leaves, flag leaves, and ears from each treatment were collected and removed at two different times during the growing season. Dates of collection were 12 July 2015, at corn reproductive stage 1 (R1), and 18 August 2015, at corn reproductive stage three (R3). On the first day of collection, it was overcast. On the second collection, it had rained in the morning before collection occurred. Collection of corn stalks, leaves, flag leaves, and ears at each time point were collected in an identical fashion.

Within each treatment, the width of the plot measured 16 plants. The length of the field, denoted as the row, from which plants were removed from the field was randomly selected at each collection time point. Collection of plants at R1 occurred from the 10^{th} row and the 68^{th} row of the corn plants for all treatments. In each desired row, the 7^{th} thru 12^{th} plant was tagged with a plastic cable tie denoting treatment and individual sample number. Corn plants were cut down, leaving 25.4 cm behind as residue in the field. All corn was removed from the field for further analysis. In total, 24 plants per treatment were collected in the field, 12 from each plot; 96 plants were collected at R1, and 96 plants were collected at R3. Collection of plants at R3 occurred in an identical manner, but from 15th row and 43th row within each treatment.

Sample measurement

Once out of the field, the mass (g) and length (m) of each sample was recorded. The length was measured from the base of the cut stalk to the tip of the flag leaf. Next, the number of total leaves on each plant, the number of green leaves on each plant, as well as, the number of yellow leaves on each plant was recorded. Disassembly of the corn stalk occurred separating the corn into four separate parts: corn ears, corn leaves, corn stalks, and corn flag leaves.

At each collection, corn ear mass (g) from each plant was recorded. Within this study, a corn ear refers to the cob and attached, intact kernels. Treatment corn ears were composited and vacuum sealed using FoodSaver V845 Vacuum Packaging System (Food Saver, Boca Raton, FL.) Samples were stored at -20°C for later nutrient analysis.

At each collection, the flag leaf from each corn plant was removed from the plant. For each treatment, twelve flag leaves were compiled and vacuum sealed using FoodSaver V845 Vacuum Packaging System (Food Saver, Boca Raton, FL.) Samples were stored at -20°C for later nutrient analysis.

At each collection, leaves from each corn plant were removed and composited. Treatment leaves were vacuum sealed using FoodSaver V845 Vacuum Packaging System (Food Saver, Boca Raton, FL). Sample were stored at -20°C for later nutrient analysis.

Lastly, at each collection, stalks from each corn plant were collected and composited. Treatment stalks were vacuum sealed using FoodSaver V845 Vacuum Packaging System (Food Saver, Boca Raton, FL). Samples were stored at -20°C for later nutrient analysis.

Nutrient analysis

After collection of plants at R1 and R3, corn leaves, corn stalks, corn flag leaves, and corn ears from the 12 original plants in the field were composited once more into one representative sample per treatment per time point to be sent for laboratory analysis (n = 16). All samples were analyzed for dietary DM, crude protein, soluble protein, NDF, ADF, fat, lignin, water soluble carbohydrates (WSC), starch, non-fibrous carbohydrates (NFC) and ash using wet chemistry at a commercial laboratory (Dairy One, http://dairyone.com/wp-content/uploads/2014/02/Forage-Lab-Analytical-Procedures-Listing-Alphabetical-July-2015.pdf, 2015).

Briefly, corn leaves, corn ears, corn stalks, and corn flag leafs were dried in force air oven at 60°C (Goering and Van Soest, 1970). For analysis of ADF, samples were individually weighted at 0.5g and digested for 75 min as a group of 24 in 2 L of ADF solution in ANKOM A200 digestion unit. Samples were rinsed three times with boiling water for 5 min in filtered bags and then soaked for 3 min in acetone, followed by drying at 105°C for 2 h (AOAC International, 2000; ANKOM, 2011). For an analysis of lignin, samples were subjected to the same treatment as ADF analysis, and residue digested as a group of 24 with 72% w/w sulfuric acid for 3 h in ANKOM Daisy incubator (AOAC International, 2000; ANKOM, 2011). For an analysis of NDF, samples were weighted at 0.5g in filter bags and digested for 75 min as a group of 24 in 2 L of NDF solution in ANKOM A200 digestion unit. Four milliter of alpha amylase and 20g of sodium sulfite were added at the start of digestion. Samples were rinsed three times with boiling water for 5 min, and alpha amylase was added in the first two rinses. After rinses, bags are soaked for 3 min in acetone, followed by drying at 105°C for 2 h (Van Soest et al., 1991; ANKOM, 2011;). Using the NRC (2001) equation for total digestible nutrients (**TDN**) and net energy for lactation (**NE$_L$**) were calculated.

Statistical analysis

Using SAS (v. 9.4, S.A.S Institute Inc., Cary NC.), data were statically analyzed as a split-plot in time design. Treatment means collected at R1 and R3 were used to make inferences about number of leaves (total, green, and yellow), the weight (corn ears and stalk), and the nutrient analysis results. Data were analyzed using the MIXED procedure of SAS by the following model:

$$Y_{ijk} = \mu + F_i + T_j + F_i \text{ x } T_j + R_k + e_{ijk},$$

where F_i = the effect of foliar fungicide treatment, T_j = the time effect of corn growth, F_i x T_j = the effect of the interaction between foliar fungicide *i* and time effect *j*, R_k = the replicate effect of *k*, and e_{ijk} is the random residual error. The model included fixed effects of treatment and time point, with random effect for replication and replication x treatment. The degree of freedom method was Kenward-Rogers (Littell et al., 1998). Results are reported as least squares means (LSM) with corresponding standard error of the mean (SEM) for fixed effects of foliar fungicide treatment. Least squares means with corresponding SEM for only significant fixed effect of time point of corn development are included in this manuscript; other values were not reported. Lignin content of the corn ear and corn leaf, phosphorus content for the corn ear and the corn flag leaf, ADF content for the corn leaf and the corn flag leaf, crude fiber content for the corn leaf, ash content for the corn leaf, crude protein content for the corn flag leaf, NDF content for the corn flag leaf, Fe content for the corn flag leaf, and Cu content for the corn flag leaf were log transformed for better distribution of values and variance of residuals. The log transformed data was back transformed and presented as LSM and SEM in tables. Treatment LSM were separated using the difference of least squares means. Significant interactions between fixed effects are presented as figures. Residuals distribution was evaluated for normality and homoscedasticity. Statistical significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. Results for non-replicated data reported as means with corresponding standard deviations (SD).

RESULTS

Corn yield

The total yield for all treatments averaged 81.3 x 10^3 kg/ha. Corn yield in CON, V5, V5+R1, and R1 totaled 78.0, 83.0, 81.3, and 82.9 x 10^3 kg/ha, respectively. During the growing season, the average daily temperature was 21.6 ± 7°C. Total rainfall in Champaign-Urbana, IL was 218.4 cm.

Soil and environmental results

Mean soil samples reported as 147.8 ± 40.8 ppm of phosphorus, 201.8 ± 44.6 ppm of potassium, 3112.2 ± 718.9 ppm of calcium, 393.1 ± 115.4 ppm of magnesium, 1.5 ± 0.17 ppm of boron, 98.0 ± 45.3 ppm of manganese, and 8.6 ± 1.9 ppm of zinc. Mean pH and buffer pH results of the soil test were 6.7 ± 0.3 and 7.0 ± 0.1, respectively. Cation exchange capacity of soil samples was 19.8 ± 4.2 mEq. Organic matter content of soil samples was 4.0 ± 0.3%.

Disease evaluation

Foliar diseases including common rust, Northern Leaf Blight, and Gray Leaf Spot were present at the R1 evaluation and the R3 evaluation. At R1, common rust was not seen on corn in CON, corn in V5, or corn in R1, and only 1% of leaf area (**LA**) of corn in V5+R1; Northern Leaf Blight was not seen on corn in V5, but was 1% of LA of corn in V5+R1, and 3% of LA of corn in CON and corn in R1; and Gray Leaf Spot was seen on corn in V5, corn in V5+R1, and corn in R1 at 1% of LA, and 2% of LA on corn in CON. At R3, common rust was not seen on corn in

CON or treated corn; Northern Leaf Blight was 2% of LA of corn in V5+R1 and corn in R1, 6% of LA of corn in V5, and 10% LA of corn in CON; Gray leaf spot was not seen on corn in R1, but was seen at 1% of LA of corn in V5+R1, 9% of LAI of corn in CON, and 15% of LA of corn in V5.

Plant measurements

Measurements of leaves, weights of stalk and ear, and height of corn stalk for corn in CON, V5, V5+R1, and R1 due to the fixed effect of treatment are in Table 3.1. No effect due to foliar fungicide treatment on corn was observed for the weight of the corn ear (P = 1.00) and weight of the corn stalk (P = 0.79). Corn in V5+R1 and corn in R1 had less yellow leaves compared with corn in CON and corn in V5 (P = 0.03). Corn in CON was shorter in height when compared with corn treated with fungicide (P = 0.01).

Measurements of leaves, weights of stalk and ear, and height of corn stalk for corn in CON, V5, V5+R1, and R1 due to the fixed effect of time are in Table 3.2. Only significant and non-interaction values are presented.

Fungicide by time point interactions were observed for the height of corn plants (Figure 3.1; P = 0.02) and the number of yellow leaves (Figure 3.2; P = 0.03). Corn in CON was shorter over the two time points than corn treated with foliar fungicide treatment. Corn in CON and in V5 had more yellow leaves over the two time points than corn in V5+R1 and corn in R1.

Corn ears

Nutrient analysis results of corn ears (cob and kernels) from corn in CON, V5, V5+R1, and R1 due to the fixed effect of treatment are in Table 3.3. No differences for fungicide treatment compared to untreated were observed for corn ears in CON, V5, V5+R1, and R1.

Nutrient analysis results of corn ears from corn in CON, V5, V5+R1, and R1 due to the fixed effect of time are in Table 3.4. Only significant and non-interaction values are presented. Starch concentration ($P < 0.0001$), and non-fibrous carbohydrates (NFC) concentration ($P < 0.0001$), increased in corn ears from R1 to R3 for corn in CON, V5, V5+R1, and R1. Interactions between foliar fungicide and time point were not observed for corn ears in CON, V5, V5+R1, R1.

Corn stalks

Nutrient analysis results of corn stalks from corn in CON, V5, V5+R1, and R1 due to the fixed effect of treatment are in Table 3.5. Corn stalks in V5+R1 resulted in greater lignin content when compared with corn stalks in CON, V5, and R1 ($P = 0.03$).

Nutrient analysis results of corn stalks from corn in CON, V5, V5+R1, and R1 due to the fixed effect of time are in Table 3.6. Only significant and non-interaction values are presented. Non-fibrous carbohydrates (NFC) concentration ($P = 0.0009$), and WSC concentration ($P = 0.0002$) were increased in corn stalks from R1 to R3 for corn in all treatments.

A fungicide by time point interaction was observed for CP content for corn stalks in CON, V5, V5+R1, R1 (Figure 3.3). Corn stalks in V5 and in R1 initially started with less CP content of at R1, but at R3 had a greater CP content when compared with corn stalks in CON and in V5+R1.

Corn leaves

Nutrient analysis results of corn leaves from corn in CON, V5, V5+R1, and R1 due to the fixed effect of treatment are in Table 3.7. No difference was observed for DM content of corn leaves ($P = 0.50$). Corn leaves in V5+R1 had a lower ADF concentration when compared with corn leaves in CON, V5, and R1 ($P = 0.01$). Corn leaves in V5+R1 had a lower NDF concentration when compared with corn leaves in CON, V5, and R1 (P = 0.02). Corn leaves in R1 had a lower Na concentration when compared with corn leaves in CON, V5, and V5+R1 (P = 0.01). Corn leaves in V5+R1 and in R1 had a greater Zn concentration when compared with corn leaves in CON and in V5 (P = 0.02). Corn leaves in V5+R1 had a lower cellulose concentration when compared with corn leaves in CON, V5, and R1 (P = 0.01).

Nutrient analysis results of corn leaves from corn in CON, V5, V5+R1, and R1 due to the fixed effect of time are seen in Table 3.8. Only significant and non-interaction values are presented. Dry matter decreased in corn leaves from R1 to R3 for corn in CON, V5, V5+R1, and R1 (P = 0.0002). Non-fibrous carbohydrates (NFC) concentration (P = 0.02), and WSC concentration (P = 0.001) increased in corn leaves from R1 to R3 for corn in CON, V5, V5+R1, and R1.

Significant foliar fungicide treatment by time point interactions for corn leaves were observed for concentration of ADF (Figure 3.4), Na (Figure 3.5), Zn (Figure 3.6), Cu (Figure 3.7), and cellulose (Figure 3.8). Corn leaves in V5+R1 had a lower ADF content over the two time points, when compared with corn leaves in CON, V5, and R1 (P = 0.008). Corn leaves in CON had the steepest decline in Na concentration when compared with treated corn leaves (P = 0.02). Corn leaves in V5+R1 and in R1 had greater concentrations of Zn over the two time points when compared with corn leaves in CON and V5 (P = 0.04). Corn leaves in V5+R1 had a lower concentration of Cu at R1, when compared to leaves in CON, V5, and R1; but the same concentration of Cu, at R3, when compared with leaves in V5+R1. No difference in cellulose concentration for corn leaves at R1 was observed, but corn leaves in V5+R1 had significantly less cellulose at R3 when compared with leaves in CON, V5, and R1.

Corn flag leaves

Nutrient analysis results of corn flag leaves from corn in CON, V5, V5+R1, and R1 due to the fixed effect of treatment are in Table 3.9. Crude fat concentration was not analyzed for corn flag leaves. Corn flag leaves in CON had greater CP concentration when compared with corn flag leaves in R1 (P = 0.03). Corn flag leaves in CON had greater concentration of Na when compared with corn flag leaves in V5, V5+R1, and R1 (P = 0.05).

Nutrient analysis results of corn flag leaves from corn in CON, V5, V5+R1, and R1 due to the fixed effect of time are in Table 3.10. Only significant and non-interaction values are presented. Dry matter content decreased in corn flag leaves from R1 to R3 for corn in CON, V5, V5+R1, and R1 (P = 0.02). Non-fibrous carbohydrates (NFC) concentration (P = 0.009), and WSC concentration (P = 0.003) increased in corn flag leaves from R1 to R3 for corn in CON, V5, V5+R1, and R1.

Significant foliar fungicide treatment by time point interaction for corn flag leaves was observed for Na concentration (Figure 3.9). At R1, no difference in concentration of Na was observed for corn flag leaves in CON, V5, V5+R1, and R1, but at R1, corn leaves in CON had significantly greater concentration of Na when compared with corn flag leaves in V5, V5+R1, and R1.

DISCUSSION

The objective of this study was to determine the effect of foliar fungicide applied at different times during the growing season of corn on the nutritive quality of corn leaves, corn ears, and corn stalks, in terms of ruminant nutrition. Because foliar fungicide is applied on the leaves of corn plants, we presumed the corn leaves would be most greatly affected.

Fungicide is applied on corn to help the crop in protection from the negative effects of fungal disease. Much of the available literature focuses on the effects of fungicide application on corn in terms of yield, both when under fungal pressure and not (Paul et al., 2011). Less literature is available on chemical changes within the corn plant when fungicide is applied to prevent diseased foliage. At the R1 disease evaluation, a total of 5% of total LA of corn in CON was infected with one of the three foliar diseases scouted. At the R3 disease evaluation, 19% of the total LA of corn in CON and 21% of the total LA in V5 was infected with at least one of the three diseases examined. Furthermore, 8.7% of the total LA of the ear leaf, the leaf adjacent to the corn cob, on corn in CON was infected with Gray Leaf Spot, compared with 1.3% of the total LA of the ear leaf in R1. During the elapsed 35 d between disease assessments, wet and cooler weather and the continued growth of the stalks and leaves may have allowed the fungus to reproduce and infect other parts of the plants. Fungicide active ingredients remain present in the waxy cuticle on the leaf for an average of 21 d post spray (Balba, 2007). After 21 d, the ingredients are considered inactive. Fungal diseases such as Gray Leaf Spot is very dependent on the weather conditions for growth and development, and transported to other leaves either by wind or water droplets (Ward et al., 1999). In the current study outbreak of disease increased greatly between the first evaluation of disease at R1 and the second evaluation for disease at R3. Application of fungicide on corn at R1 may have been active on the leaves of corn in R1 and in V5+R1, allowing for better prevention of blighted tissue when the increase in disease outbreak occurred.

In a similar way, corn plants in V5+R1 and R1 had less yellow leaves at R3 compared with V5 and CON (Figure 3.2). Numerically, corn plants in V5+R1 and R1 had a greater amount of green leaves at R3 (Table 3.2). Ruske et al. (2003) reported that applications of foliar fungicide on wheat delayed senesce of leaves, measured by the amount of green area on the flag leaf, and allowed for better control of disease. Delaying senesce by 1 wk was associated with a grain yield increase of 0.9 x 10^3 kg/ha every week it is was delayed (Ruske et al., 2003). Application of fungicide at R1 may have been crucial in the prevention of infection from fungal pathogens, reducing the blighted tissue and increasing the green color. Less photosynthetic area on the leaves of corn and redirection of photosynthate in CON may have caused wilting of the plant. At R3, corn in V5+R1 was 0.5 m taller than corn in CON (Figure 3.1). In a year when outbreak of Gray Leaf Spot was severe, Roane et al. (1974) observed extreme breakage and stalk rot in crops. In this study, we hypothesize not all plants in CON at the R3 collection were suffering from lodging and stalk rot, but possibly greater wilting as the visible disease lesions for Gray Leaf Spot observed was the most for corn in CON. Diseases such as Northern Leaf Blight (Dodd, 1980) and Gray Leaf Spot (Ward et al., 1999) can cause premature wilting and gradual loss of leaves. Lignin concentration of the corn stalks was also greater for corn in V5+R1 when compared with CON (Table 3.5). Lignin comprises about 40 to 60% of the cell wall (Jung, 2012) and structurally gives plant's there shape and rigidity. A 33-unit increase in the concentration of lignin in the stalks of corn in V5+R1 at R3 may have been why corn in V5+R1 were taller when compared with stalks in CON (Figure 3.1). As grasses mature, the lignin content in the stem increases (Mowat et al., 1969). When feeding ruminants, increasing lignin content of a feedstuff was negatively correlated with the ruminal digestibility (Mowat et al., 1969; Hunt et al., 1992).

There were no treatment differences in the nutritive quality of the corn ears due to the effect of foliar fungicide applications (Table 3.3). Because the primary target for foliar fungicide applications is the leaves (Balba, 2007), the local protection of the application may not affect the cob and grain content. Had physical damage occurred to the corn kernels, in the form of insects, disease, or hail, damaged corn would have been expected to have a lower concentration of starch (Teller et al., 2012). Furthermore, Roth and Lauer (2008) observed that defoliation at the R1 resulted in a lower grain yield than defoliation at V7, V10, or R3. In the current study, results for starch and WSC concentration in the corn ear, which included the cob and intact kernels, could have been different if severe fungal growth occurred at tassel or R1, limiting the tissue to available photosynthesize. Wang et al. (2010) reported lower DM content for corn grain from severely diseased corn plants when Northern Leaf Blight lesions were visible on 80 to 95% of the total leaf area. As expected, during the reproductive phase of corn growth, the concentration of grain DM increased for all treatments (Table 3.4). Weaver et al. (1978) also reported an increase in grain DM of corn as the plant matured.

As hypothesized, foliar fungicide caused the most nutritive effects in the corn leaf content. Before collection of the leaves at R3 it had rained and may be the reason the DM decreased for all treatments at R3 (Table 3.8). As corn plant matures, the DM of the plant increases, as the leaves begin to dry down and direct nutrients to the ear completing grain-filling process. Weaver et al. (1978) observed decreases in the DM content of the leaves as the plant advanced in maturity, attributing it to increases in moisture from snow and rain; all other parts of the plant were unaffected by the increase in moisture content. Corn leaves in V5+R1 had less ADF and NDF when compared with CON and V5 (Table 3.7). Wang et al. (2010) reported that severely diseased corn plants had greater ADF and NDF concentrations. The greater amount of disease seen on the foliar leaves at the R3 evaluation and the lack of active protection from fungicide may have increased the fibrous content within the leaves. By preventing fungal degradation with an application of foliar fungicide at R1, corn leaves in V5+R1, and possibly R1, resulted in less fibrous content. In a two-year study, Johnson et al. (1997) reported an increased ADF content of 6.9 units in the DM of diseased corn when compared to the previous year when no disease was visible. The authors attributed the increased ADF concentration to be the result of 30.2% of total LA was severely diseased.

A significant interaction occurred for the ADF content of the leaf and the advancing maturity of the corn plant (Figure 3.4). It appears that corn leaves in V5+R1 had a lower concentration of ADF when compared with the other leaves (Table 3.7). This may be due to applications of the foliar fungicide preventing fungal disease and decreasing the fibrous content within the tissue. Yet, the apparent decrease in fibrous concentration of corn leaves in V5+R1 may also be the result of increased concentration of non-fibrous carbohydrates content within the leaves, 46 units greater than leaves in CON (Table 3.7). We think it may be a slight overestimation of the decrease in fibrous content of the leaves in V5+R1, as the sugar content is increasing as the stalks mature. Corn leaves in V5+R1 had an 83 unit increase in the concentration of NFC at R3 when compared with CON (Table 3.8). Additionally, corn leaves in R1 resulted in increased NFC concentration 25 units greater than corn leaves in CON (Table 3.8). Application of fungicide on corn at R1 may have increased the sugar content within the plant. Fungicide applications on corn at the silking stage (R1) have been shown to significantly increase grain yield compared to untreated corn (Blandino et al., 2012; Testa et al., 2015). Haerr et al. (2015) fed dairy cows with corn silage treated with various applications of foliar fungicide, observing a positive linear response of feed conversion to increasing number of fungicide applications. Cows fed corn silage from corn where at least one of the fungicide applications occurred at R1 had numerically increased feed conversion of dry matter to milk when compared to cows fed control and cows fed corn silage from corn with only one application of fungicide at V5 (Haerr et al., 2015). The authors attributed part of the increased feed conversion to be the result of increased sugar content from corn silage with fungicide application (Haerr et al., 2015).

At R1, concentration of Na of corn leaves in CON was the greatest compared to leaves in V5, V5+R1, and R1 (Figure 3.5). Furthermore over the two time points, the Na content within the flag leaves increased the most for CON compared with fungicide treated corn (Figure 3.9). Wu and von Tiedemann (2001) performed an experiment evaluating fungicide application on wheat oxidative stress. The authors reported a greater concentration of the leakage of ions from leaves when fungicide was not applied compared with when fungicide was applied. It was hypothesized that fungicide application delays senescence of the leaves by limiting electrolyte leakage, as electrolyte leakage is an early symptom of leaf senescence (Wu and von Tiedemann, 2001). Corn leaves in V5, V5+R1, and R1 had greater Cu concentration at R3 compared with corn leaves in CON (Figure 3.7). Copper in the plant is a micronutrient essential for growth and development of the plant. Copper ions act as cofactors for many enzymes, including superoxidase dismutase (Yruela, 2005) located in the cell wall, cytosol, chloroplasts, and peroxisome of plant cells. (Alscher et al., 2002). Superoxide dismustase converts reactive O_2 species into oxygen or peroxide, which can originate when the plant cell is stressed. Perhaps, applications of fungicide on corn increase the antioxidant capacity of the plant. More research on this topic is needed.

It is unknown how the physiological benefits of fungicide on corn plants individually impact the fermentation and digestibility of each part of the corn plant within ruminants. Results from our study indicate applications of foliar fungicide at both V5 and R1 together on corn reduced ADF and NDF concentration within the leaves (Table 3.7), but increased lignin content within the stalk (Table 3.5). *In situ* correlation coefficients for the ruminal fermentability of whole plant corn samples has been negatively correlated with lignin, NDF, and ADF (Hunt et al., 1992). Li et al. (2014) evaluated the *in-situ* degradability of whole corn stover and seven different morphological fractions apart of corn stover using Chinese-Holsteins. Neutral detergent fiber, ADF, and DM were most degradable for the leaf blades and stem pith, followed by ear husk, but DM disappearance after 48 h in the rumen was lowest for stem node and rind (Li et al., 2014). The authors suggested increased lignin content within the stalk of the corn plant may limit digestibility. In an analysis of 96 dietary treatments, a 0.01 increase in NDF organic matter digestibility increased milk yield 0.08 kg, DMI 0.02 kg, and milk lactose 0.02 kg (Krämer-Schmid et al., 2016). Wang et al. (2010) did not observe differences in the digestibility of ADF or NDF when highly diseased corn silage was fed to sheep compared to sheep fed control corn silage, although a difference in DM digestibility was reported. More research is needed to determine the effects of decreasing the fibrous part of one part of the plant, while increasing the fibrous part of another, on microbial metabolism of the feedstuff within the rumen, especially when each part of the corn plant makes up a different proportion of corn silage.

CONCLUSIONS

Applications of fungicide on corn under disease pressure resulted in less yellow leaves, and taller corn plants when compared with corn that did not receive fungicide application. Fungicide application on corn at both V5 and R1 reduced the NDF and ADF concentration of the leaves, and increased the lignin concentration of the stalks when compared with untreated corn plants. Fungicide application on corn may reduce the impacts of fungal stress and reduce the fibrous content within the plant. For ruminant nutritionists and producers, silage made from fungicide treated corn may reduce the bulk of the forage and enhance the quality of the feedstuff.

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TABLES AND FIGURES

Table 3.1. Least squares means and associated standard errors for physical measurements of corn in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]					*P*-value
						Fixed effects[2]
	CON	**V5**	**V5+R1**	**R1**	**SEM**	**TRT**
Number of total leaves	12.0	12.2	12.6	12.1	0.15	0.21
Number of yellow leaves	0.85^{a}	0.77^{a}	0.42^{b}	0.44^{b}	0.07	0.03
Number of green leaves	11.2	11.4	12.1	11.6	0.21	0.12
Weight of stalk, g	989	1059	1071	1088	72.7	0.79
Weight of ear, g	184	185	184	185	6.1	1.00
Height of stalk, m	2.7^{c}	2.9^{ab}	3.0^{a}	2.9^{ab}	0.03	0.01

[1] Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2] Fixed effects of (TRT) effect due to fungicide treatment on corn plants.

Table 3.2. Least squares means and associated standard errors for physical measurements at the first collection (R1) and the second collection (R3) of corn in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]									***P*-value**
	CON		**V5**		**V5+R1**		**R1**			**Fixed effects**[2]
	R1	**R3**	**R1**	**R3**	**R1**	**R3**	**R1**	**R3**	**SEM**	**TP**
Number of total leaves	13.0	11.0	13.1	11.3	13.4	11.7	13.3	10.9	0.22	0.0002
Number of green leaves	13.0	9.3	13.1	9.8	13.4	10.9	13.3	10.0	0.30	0.0001
Weight of stalk, g	745	1233	717	1340	810	1332	863	1313	102.8	0.002
Weight of ear, g	27	340	31	338	21	347	31	339	8.6	<0.0001

[1] Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2] Fixed effects of (TP) effect due to stage of vegetative growth when collected.

Figure 3.1. Height of stalk for corn stalk in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction $P = 0.02$.

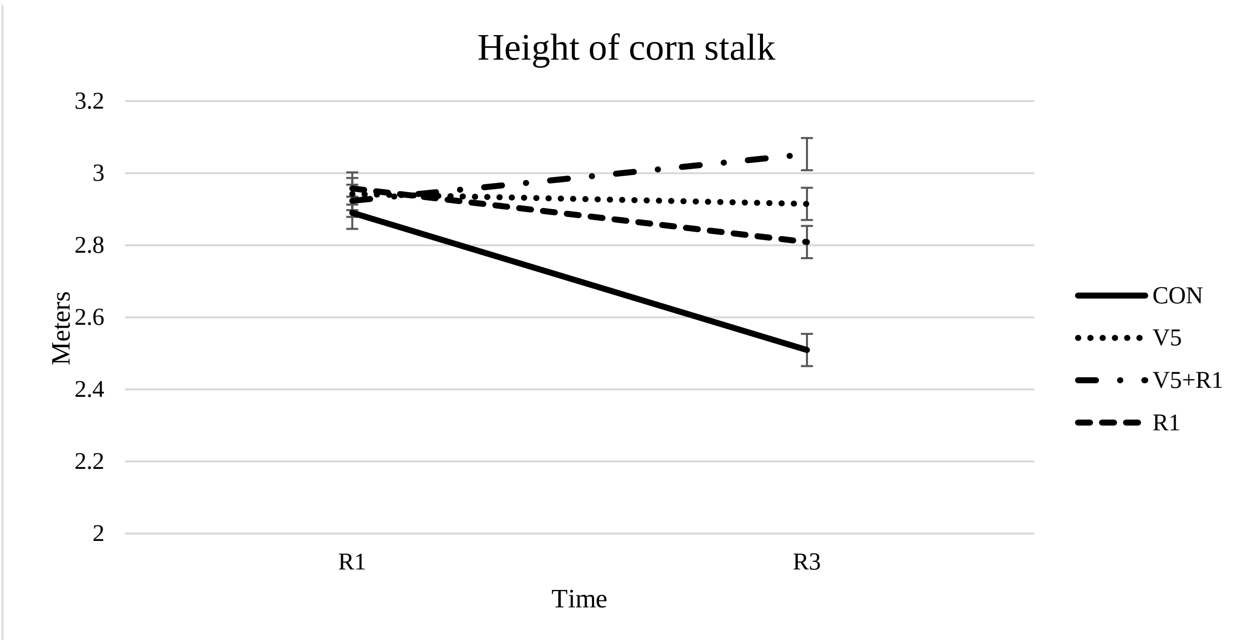


Figure 3.2. Number of yellow leaves on corn in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction $P = 0.03$.

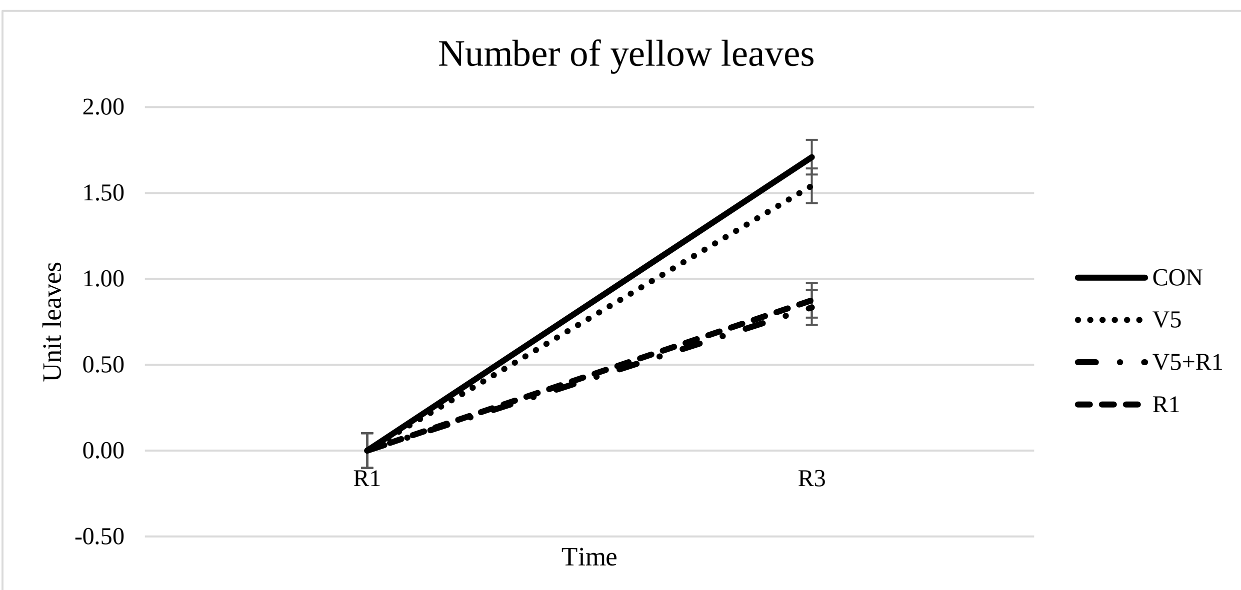


Table 3.3. Least squares means and associated standard errors for corn ears in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]					***P*-value**
						Fixed effects[2]
	CON	**V5**	**V5+R1**	**R1**	**SEM**	**TRT**
Corn ears composition						
DM, g/kg	269	270	264	283	10.0	0.61
CP, g/kg DM	253	250	267	263	13.7	0.79
Soluble CP, g/kg of CP	453	448	428	475	16.0	0.34
ADF, g/kg DM	114	120	120	118	4.2	0.68
NDF, g/kg DM	196	193	185	196	8.0	0.75
Lignin, g/kg DM[3]	28	12	17	11	9.0	0.52
NFC, g/kg DM	417	427	409	407	13.7	0.75
Starch, g/kg DM	318	331	324	313	6.1	0.31
WSC, g/kg DM[4]	71	86	79	90	6.8	0.36
Crude Fat, g/kg DM	69	66	75	65	4.4	0.49
Ash, g/kg DM	65	65	64	69	3.9	0.81
Ca, g/kg DM	1.7	1.7	1.7	1.8	0.1	0.87
P, g/kg DM[3]	7.0	7.1	7.1	7.6	0.4	0.81
Mg, g/kg DM	2.5	2.5	2.6	2.7	0.2	0.91
K, g/kg DM	23.1	23.7	23.4	25.5	1.5	0.69
Na, g/kg DM	0.07	0.04	0.04	0.04	0.009	0.21
S, g/kg DM	3.2	3.2	3.4	3.4	0.1	0.74
Fe, ppm	83.50	67.75	108.00	105.25	8.90	0.09
Zn, ppm	67.25	68.75	81.00	73.00	4.97	0.33
Cu, ppm	11.25	10.75	12.00	11.25	0.78	0.74
Mn, ppm	31.25	28.50	31.50	34.00	2.20	0.46
Molydbenum, ppm	0.40	0.35	0.48	0.45	0.07	0.61
Hemicellulose, g/kg DM[5]	82.5	73.3	64.8	78.3	7.4	0.46
Cellulose, g/kg DM[6]	86.0	108.0	103.3	106.8	12.0	0.59
Energy Calculations[7]						
NE_l, MJ/kg	8.26	8.39	8.51	8.37	0.17	0.77
NE_g, MJ/kg	5.95	6.09	6.18	6.06	0.17	0.80
NE_m, MJ/kg	8.74	8.86	9.02	8.83	0.20	0.81
TDN, g/kg DM	82.75	84.00	84.50	83.75	1.43	0.85

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2] Fixed effects of (TRT) effect due to fungicide treatment on corn plants with superscripts denoting statistical differences.

(Table 3.3 continued)
[3]Log transformed data, back transformed units presented.
[4]Water soluble carbohydrates.
[5]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.
[6]Cellulose, g/kg DM = ADF, g/kg DM – Lignin, g/kg DM.
[7]NRC(2001).

Table 3.4. Least squares means and associated standard errors at the first collection (R1) and the second collection (R3) for corn ears in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]								SEM	*P*-value Fixed effects[2]
	CON		V5		V5+R1		R1			
	R1	R3	R1	R3	R1	R3	R1	R3		TP
Corn ears composition										
DM, g/kg	55	483	51	490	44	484	59	508	14.1	<0.0001
CP, g/kg DM	435	75	421	78	458	77	442	84	19.3	<0.0001
Soluble CP, g/kg of CP	555	350	540	350	515	340	575	375	22.6	0.0003
ADF, g/kg DM	144	84	163	77	158	83	154	81	5.9	<0.0001
NDF, g/kg DM	198	195	219	167	194	177	208	184	11.3	0.04
NFC, g/kg DM	151	683	153	701	127	692	136	679	19.4	<0.0001
Starch, g/kg DM	42	595	26	636	32	615	18	607	8.6	<0.0001
WSC, g/kg DM[3]	77	66	102	70	81	78	111	69	9.6	0.03
Crude Fat, g/kg DM	108	31	96	36	112	38	94	37	6.2	0.0001
Ash, g/kg DM	112	18	112	18	111	16	120	17	5.5	<0.0001
Ca, g/kg DM	3.3	0.1	3.2	0.1	3.3	0.1	3.5	0.1	0.2	<0.0001
P, g/kg DM[3]	1.1	0.3	1.1	0.3	1.1	0.3	1.2	0.3	0.06	<0.0001
Mg, g/kg DM	4.1	0.9	4.1	1.0	4.2	1.0	4.4	1.0	0.3	0.0001
K, g/kg DM	4.1	0.6	4.2	0.5	4.2	0.5	4.6	0.5	0.21	<0.0001
Na, g/kg DM	0.1	0.04	0.05	0.03	0.04	0.04	0.05	0.04	0.01	0.05
S, g/kg DM	5.4	1.1	5.4	1.1	5.7	1.1	5.6	1.2	0.2	<0.0001
Zn, ppm	114.5	20.0	117.0	20.5	140.5	21.5	123.5	22.50	7.03	<0.0001
Cu, ppm	19.5	3.0	18.5	3.0	21.0	3.0	20.0	2.50	1.10	<0.0001
Mn, ppm	57.5	5.0	52.0	4.5	58.0	5.0	63.0	5.00	3.11	<0.0001
Hemicellulose, g/kg DM[5]	54	111	57	90	36	94	54	103	10.5	0.003
Cellulose, g/kg DM[6]	97	75	151	66	136	71	142	72	17.0	0.007

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2]Fixed effects of (TP) effect due to stage of vegetative growth when collected.
[3]Water soluble carbohydrates.
[4]Log transformed data, back transformed units presented.
[5]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.
[6]Cellulose, g/kg DM = ADF, g/kg DM – Lignin, g/kg DM.

Table 3.5. Least squares means and associated standard errors for corn stalks in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]					***P*-value**
	CON	**V5**	**V5+R1**	**R1**	**SEM**	**Fixed effects**[2] **TRT**
Corn stalks composition						
DM, g/kg	160	163	158	162	6.5	0.95
CP, g/kg DM	72	75	73	73	1.4	0.67
Soluble CP, g/kg of CP	713	693	668	658	20.8	0.36
ADF, g/kg DM	449	479	489	471	11.3	0.23
NDF, g/kg DM	646	667	674	680	15.8	0.53
Lignin, g/kg DM	46^{b}	56^{ab}	64^{a}	50^{b}	2.7	0.03
NFC, g/kg DM	207	178	182	171	14.7	0.44
Starch, g/kg DM	16	7	11	4	4.8	0.42
WSC, g/kg DM[3]	133	132	128	135	13.0	0.98
Crude Fat, g/kg DM	9.0	9.0	10.5	9.3	0.6	0.33
Ash, g/kg DM	67	72	61	68	3.3	0.26
Ca, g/kg DM	2.1	2.1	2.0	2.1	0.1	0.91
P, g/kg DM	2.4	2.3	2.1	2.3	0.1	0.67
Mg, g/kg DM	1.7	1.7	1.7	1.6	0.2	0.91
K, g/kg DM	27.9	26.0	24.9	27.0	1.6	0.97
Na, g/kg DM	0.06	0.05	0.05	0.03	0.009	0.64
S, g/kg DM	0.9	0.9	0.8	0.9	0.04	0.85
Fe, ppm	155.50	151.00	172.75	179.75	24.97	0.82
Zn, ppm	23.75	24.25	26.00	24.75	2.20	0.90
Cu, ppm	6.00	5.75	5.50	5.25	0.59	0.83
Mn, ppm	18.75	16.75	15.75	19.75	2.44	0.67
Molydbenum, ppm	0.45	0.50	0.40	0.48	0.08	0.81
Hemicellulose, g/kg DM[4]	197	188	185	208	5.8	0.14
Cellulose, g/kg DM[5]	403	423	425	421	9.4	0.45
Energy Calculations[6]						
NE_l, MJ/kg	4.50	4.15	4.04	4.10	0.16	0.32
NE_g, MJ/kg	2.35	2.01	1.94	2.14	0.10	0.12
NE_m, MJ/kg	4.66	4.27	4.24	4.43	0.12	0.19
TDN, g/kg DM	580	553	550	563	7.3	0.13

(Table 3.5 continued)

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).

[2]Fixed effects of (TRT) effect due to fungicide treatment on corn plants with superscripts denoting statistical differences.

[3]Water soluble carbohydrates.

[4]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.

[5]Cellulose, g/kg DM = ADF, g/kg DM – Lignin, g/kg DM.

[6]NRC(2001).

Table 3.6. Least squares means and associated standard errors at the first collection (R1) and the second collection (R3) for corn stalks in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]								SEM	*P*-value
	CON		V5		V5+R1		R1			Fixed effects[2]
	R1	R3	R1	R3	R1	R3	R1	R3		TP
Corn stalks composition										
Soluble CP, g/kg of CP	790	635	790	595	830	505	795	520	29.4	0.0003
NDF, g/kg DM	645	637	695	639	712	636	730	695	22.4	0.02
Lignin, g/kg DM	39	52	51	61	56	72	51	50	3.8	0.03
NFC, g/kg DM	161	252	121	235	107	258	90	252	20.8	0.0009
WSC, g/kg DM[3]	51	216	51	213	51	205	39	231	18.4	0.0002
Crude Fat, g/kg DM	11	8	11	7	13	8	13	6	0.8	0.0013
Ash, g/kg DM	83	50	81	63	74	48	81	54	4.6	0.0014
P, g/kg DM	3	2	3	2	3	2	3	2	0.2	0.0007
Mg, g/kg DM	2	1	2	1	2	2	2	1	0.2	0.03
K, g/kg DM	33	23	30	22	31	19	29	25	2.3	0.0072
S, g/kg DM	1.0	0.8	1.0	0.7	1.0	0.7	1.0	0.8	0.06	0.002
Zn, ppm	31.00	16.50	29.00	19.50	32.50	19.50	30.00	19.50	3.12	0.006
Hemicellulose, g/kg DM[4]	205	189	210	161	217	153	228	189	8.2	0.002
Cellulose, g/kg DM[5]	411	396	428	418	439	411	451	392	13.3	0.04
Energy Calculations[6]										
NE_l, MJ/kg	4.38	4.61	3.87	4.43	3.69	4.38	3.46	4.75	0.23	0.01
NE_g, MJ/kg	2.26	2.44	1.89	2.12	1.80	2.08	1.80	2.54	0.14	0.02
NE_m, MJ/kg	4.57	4.75	4.10	4.43	4.10	4.38	4.01	4.84	0.17	0.03
TDN, g/kg DM	570	590	545	560	545	555	535	590	1.03	0.03

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2]Fixed effects of (TP) effect due to stage of vegetative growth when collected.
[3]Water soluble carbohydrates.
[4]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.
[5]Cellulose, g/kg DM = ADF, g/kg DM – Lignin, g/kg DM.
[6]NRC(2001).

Figure 3.3. Crude protein, g/kg DM, for corn stalks in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction $P = 0.04$.

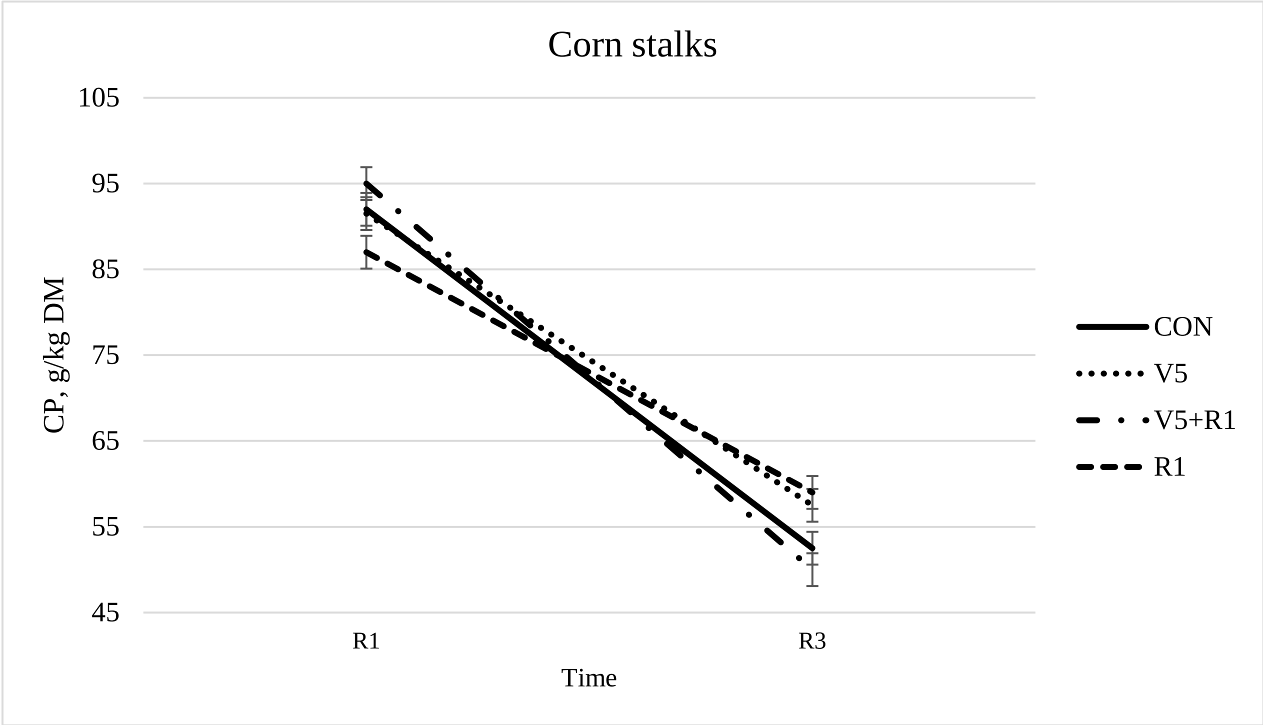


Table 3.7. Least squares means and associated standard errors for corn leaves in (CON), one application of foliar fungicide at V5 (V5), one application of foliar fungicide at R1 (R1), or two applications of foliar fungicide at V5 and R1 (V5+R1).

	Treatment[1]					*P*-value
						Fixed effects[2]
	CON	V5	V5+R1	R1	SEM	TRT
Corn leaves composition						
DM, g/kg DM	196	202	193	194	4.1	0.50
CP, g/kg DM	171	162	161	164	2.8	0.18
Soluble CP, g/kg of CP	465	450	468	473	1.19	0.62
ADF, g/kg DM[3]	333[a]	331[ab]	283[d]	330[abc]	6.2	0.01
NDF, g/kg DM	569[ab]	584[a]	524[d]	554[bc]	7.4	0.02
Lignin, g/kg DM[3]	20	18	18	17	1.8	0.81
NFC, g/kg DM	62	61	108	85	12.5	0.15
Starch, g/kg DM	5	7	5	7	1.5	0.77
WSC, g/kg DM [4]	44	46	49	52	4.9	0.73
Crude Fat, g/kg DM[3]	45	42	41	43	1.0	0.26
Ash, g/kg DM[3]	154	151	168	155	10.7	0.69
Ca, g/kg DM	7.3	8.7	7.7	7.2	0.4	0.26
P, g/kg DM	4.2	4.0	4.1	4.3	0.1	0.32
Mg, g/kg DM	3.0	3.2	3.0	2.8	0.2	0.58
K, g/kg DM	22.8	22.7	23.6	24.3	0.4	0.14
Na, g/kg DM	0.1[a]	0.08[bc]	0.08[ab]	0.06[d]	0.005	0.01
S, g/kg DM	2.0	2.0	2.0	2.1	0.04	0.41
Fe, ppm	1180.50	1094.75	1447.75	1167.00	232.14	0.73
Zn, ppm	32.00[b]	30.75[b]	36.50[a]	36.50[a]	0.91	0.02
Cu, ppm	13.25	13.50	14.00	14.75	0.31	0.09
Mn, ppm	83.50	77.50	92.50	84.50	8.31	0.67
Molydbenum, ppm	1.53	1.40	1.53	1.50	0.16	0.94
Hemicellulose, g/kg DM [5]	236	253	241	235	4.5	0.13
Cellulose, g/kg DM[6]	315[a]	314[ab]	265[d]	303[abc]	6.3	0.01
Energy Calculations[7]						
NE_l, MJ/kg	5.33	5.26	5.42	5.44	0.14	0.78
NE_g, MJ/kg	3.00	2.93	2.93	3.04	0.17	0.95
NE_m, MJ/kg	5.37	5.33	5.30	5.42	0.19	0.97
TDN, g/kg DM	608	610	600	615	11.5	0.83

(Table 3.7 continued)

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).

[2]Fixed effects of (TRT) effect due to fungicide treatment on corn plants with superscripts denoting statistical differences.

[3]Log transformed data, back transformed units presented.

[4]Water soluble carbohydrates.

[5]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.

[6]Cellulose, g/kg DM = ADF, g/kg DM – Lignin, g/kg DM.

[7]NRC(2001).

Table 3.8. Least squares means and associated standard errors at the first collection (R1) and the second collection (R3) for corn leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]									***P*-value**
	CON		**V5**		**V5+R1**		**R1**		**SEM**	**Fixed effects**[2]
	R1	**R3**	**R1**	**R3**	**R1**	**R3**	**R1**	**R3**		**TP**
Corn leaves composition										
DM, g/kg DM	229	164	234	170	209	178	216	172	5.7	0.0002
CP, g/kg DM	206	136	191	133	183	138	195	133	3.9	<0.0001
Soluble CP, g/kg of CP	610	320	580	320	645	290	620	325	16.8	<0.0001
NDF, g/kg DM	582	556	588	581	594	434	579	530	10.5	0.002
Lignin, g/kg DM[3]	26	14	20	16	18	18	21	13	2.6	0.03
NFC, g/kg DM	54	72	47	75	59	158	70	100	17.6	0.02
WSC, g/kg DM [4]	28	60	23	69	30	69	30	73	6.9	0.001
Crude Fat, g/kg DM[3]	54	37	49	36	44	38	45	40	1.4	0.0009
Ash, g/kg DM[3]	107	200	125	177	122	214	112	197	15.2	0.001
Ca, g/kg DM	5.9	8.8	6.9	10.5	6.2	9.2	6.3	8.2	0.7	0.004
P, g/kg DM	4.5	4.0	4.4	3.7	4.1	4.1	4.6	4.1	0.1	0.01
Mg, g/kg DM	2.5	3.5	2.8	3.5	2.6	3.4	2.7	2.9	0.3	0.02
S, g/kg DM	2.3	1.8	2.1	1.9	2.1	1.9	2.2	2.0	0.06	0.003
Fe, ppm	311.00	2050.00	554.50	1635.00	500.50	2395.00	414.00	1920.00	328.30	0.003
Mn, ppm	46.00	121.00	58.50	96.50	53.00	132.00	55.50	113.50	11.75	0.002
Hemicellulose, g/kg DM[5]	253.0	218.5	252.5	253.0	255.5	226.0	242.5	226.5	6.3	0.01
Energy Calculations[6]										
NE_l, MJ/kg	5.67	4.98	5.53	4.98	5.44	5.40	5.63	5.26	0.20	0.04
NE_g, MJ/kg	3.55	2.44	3.32	2.54	3.32	2.54	3.41	2.68	0.24	0.007
NE_m, MJ/kg	6.00	4.75	5.76	4.89	5.72	4.89	5.81	5.03	0.26	0.008
TDN, g/kg DM	650	565	640	580	635	565	645	585	16.3	0.004

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2]Fixed effects of (TP) effect due to stage of vegetative growth when collected.
[3]Log transformed data, back transformed units presented.
[4]Water soluble carbohydrates.
[5]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.
[6]NRC(2001).

Table 3.9. Least squares means and associated standard errors for corn flag leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]					***P*-value**
						Fixed effects[2]
	CON	**V5**	**V5+R1**	**R1**	**SEM**	**TRT**
Corn flag leaves composition						
DM, g/kg DM	274	298	292	268	11.5	0.34
CP, g/kg DM[3]	144^{a}	144^{ab}	135^{abc}	128^{c}	2.6	0.03
Soluble CP, g/kg of CP	505	488	528	503	28.6	0.80
ADF, g/kg DM[3]	355	353	368	360	6.6	0.44
NDF, g/kg DM[3]	595	580	593	596	15.7	0.86
Lignin, g/kg DM	9	9	10	8	1.7	0.84
NFC, g/kg DM	81	72	78	83	11.2	0.91
Starch, g/kg DM	5	3	3	5	0.7	0.13
WSC, g/kg DM [4]	47	47	51	43	4.8	0.74
Ca, g/kg DM	8.3	9.6	8.4	7.5	1.1	0.63
P, g/kg DM[3]	03.7	03.7	03.6	03.3	0.1	0.33
Mg, g/kg DM	2.5	02.8	02.6	02.1	0.3	0.46
K, g/kg DM	13.3	14.0	14.5	14.9	0.6	0.45
Na, g/kg DM	0.08^{a}	0.04^{b}	0.04^{b}	0.05^{b}	0.006	0.05
S, g/kg DM	2.9	2.7	2.6	2.6	0.2	0.58
Fe, ppm[3]	1160	1566	1625	1435	415	0.93
Zn, ppm	112.00	90.00	99.00	95.25	9.06	0.46
Cu, ppm[3]	25.50	21.50	21.50	19.50	1.32	0.24
Mn, ppm	127.75	133.00	123.00	137.50	20.47	0.96
Molydbenum, ppm	1.43	1.28	1.40	1.33	0.18	0.90
Hemicellulose, g/kg DM[5]	240	228	225	236	10.0	0.72
Cellulose, g/kg DM[6]	346	343	358	352	7.4	0.57
Energy Calculations7						
NE_l, MJ/kg	4.77	4.77	4.80	4.73	0.12	0.98
NE_g, MJ/kg	2.70	2.61	2.68	2.63	0.22	0.99
NE_m, MJ/kg	5.05	4.98	5.05	5.03	0.24	1.00
TDN, g/kg DM	598	588	595	595	17.9	0.98

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2]Fixed effects of (TRT) effect due to fungicide treatment on corn plants with superscripts denoting statistical differences.
[3]Log transformed data, back transformed units presented.
[4]Water soluble carbohydrates.

(Table 3.9 continued)
[5]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.
[6]Cellulose, g/kg DM = ADF, g/kg DM – Lignin, g/kg DM.
[7]NRC(2001).

Table 3.10. Least squares means and associated standard errors at the first collection (R1) and the second collection (R3) for corn flag leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatment[1]									*P*-value
	CON		V5		V5+R1		R1		SEM	Fixed effects[2]
	R1	R3	R1	R3	R1	R3	R1	R3		TP
Corn flag leaves composition										
DM, g/kg DM	296	252	337	259	305	279	276	260	16.2	0.02
CP, g/kg DM[3]	165	124	167	121	160	110	149	108	3.7	<0.0001
Soluble CP, g/kg of CP	620	390	565	410	595	460	580	425	40.4	0.004
ADF, g/kg DM[3]	391	320	387	319	397	339	390	331	9.4	0.0006
NDF, g/kg DM[3]	701	489	698	463	707	479	710	483	22.2	0.0002
Lignin, g/kg DM	13	6	11	8	14	7	9	7	2.5	0.06
NFC, g/kg DM	54	108	54	91	49	108	52	115	15.8	0.009
WSC, g/kg DM[4]	31	64	31	63	36	66	28	59	6.8	0.003
Ca, g/kg DM	2.3	14.3	2.8	16.3	2.3	14.6	2.3	12.7	1.5	0.0004
P, g/kg DM[3]	4.3	3.1	4.5	2.9	4.4	2.8	4.1	2.5	0.2	0.0005
Mg, g/kg DM	1.9	3.1	2.1	3.5	1.8	3.3	1.7	2.6	0.4	0.008
K, g/kg DM	17.4	9.3	18.0	10.1	18.9	10.1	18.1	11.7	0.9	0.0003
S, g/kg DM	2.6	3.2	2.6	2.9	2.4	2.9	2.2	2.9	0.2	0.03
Fe, ppm[3]	440	1880	367	2765	375	2875	479	2390	587	0.0007
Zn, ppm	60.00	164.00	61.50	118.50	58.00	140.00	57.50	133.00	12.81	0.0009
Cu, ppm[3]	7.50	43.50	7.50	35.50	6.50	36.50	7.00	32.00	1.87	<0.0001
Mn, ppm	39.00	216.50	45.50	220.50	41.00	205.0	49.00	226.00	28.94	0.001
Molydbenum, ppm	0.40	2.45	0.55	2.00	0.40	2.40	0.25	2.40	0.26	0.0005
Hemicellulose, g/kg DM[5]	310.5	169.5	311	145	310	141	320	152	14.2	<0.0001
Cellulose, g/kg DM[6]	378	314	376	311	383	332	381	324	10.5	0.001
Energy Calculations[7]										
NE_g, MJ/kg	3.46	1.94	3.41	1.80	3.46	1.89	3.37	1.89	0.31	0.002
NE_m, MJ/kg	5.86	4.24	5.90	4.06	5.90	4.20	5.81	4.24	0.35	0.002
TDN, g/kg DM	665	530	665	510	665	525	660	530	25.4	0.002

[1]Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application of fungicide at V5), R1 (with 1 application of fungicide at R1), and V5+R1 (with 2 applications of fungicide at V5 and R1).
[2]Fixed effects of (TP) effect due to stage of vegetative growth when collected.
[3]Log transformed data, back transformed units presented.
[4]Water soluble carbohydrates.
[5]Hemicellulose, g/kg DM = NDF, g/kg DM – ADF, g/kg DM.
[6]Cellulose, g/kg DM = ADF, g/kg DM – Lignin, g/kg DM.

(Table 3.10 continued)
[7]NRC(2001).

Figure 3.4. Acid detergent fiber, g/kg DM, for corn leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction *P* = 0.008.

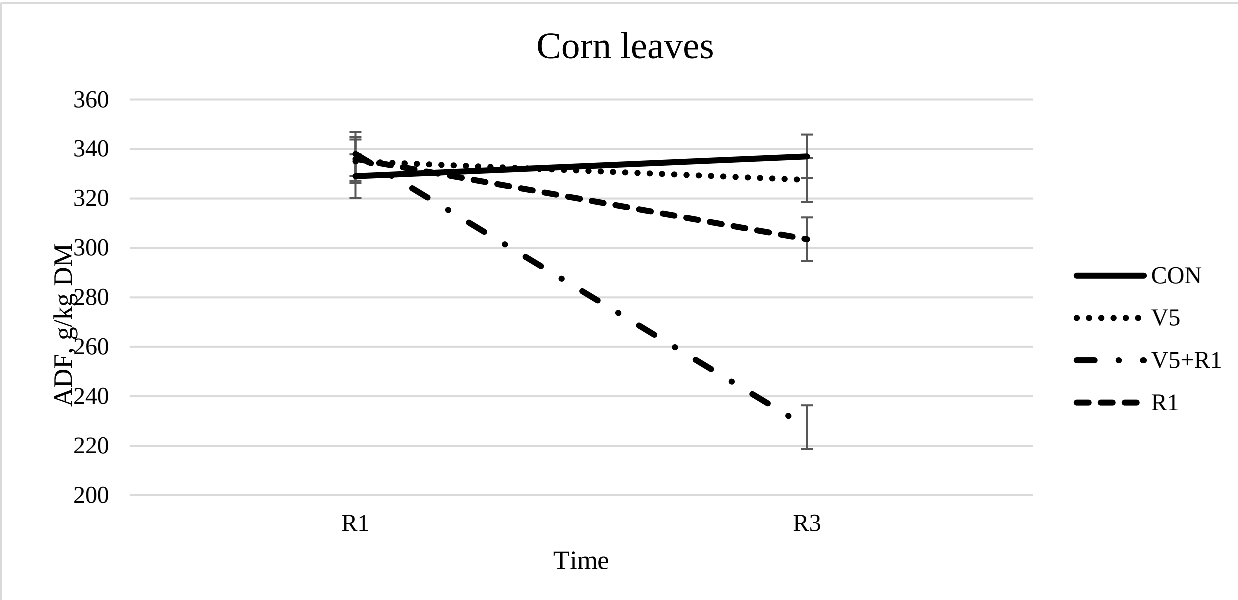


Figure 3.5. Sodium, g/kg DM, for corn leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction *P* = 0.02.

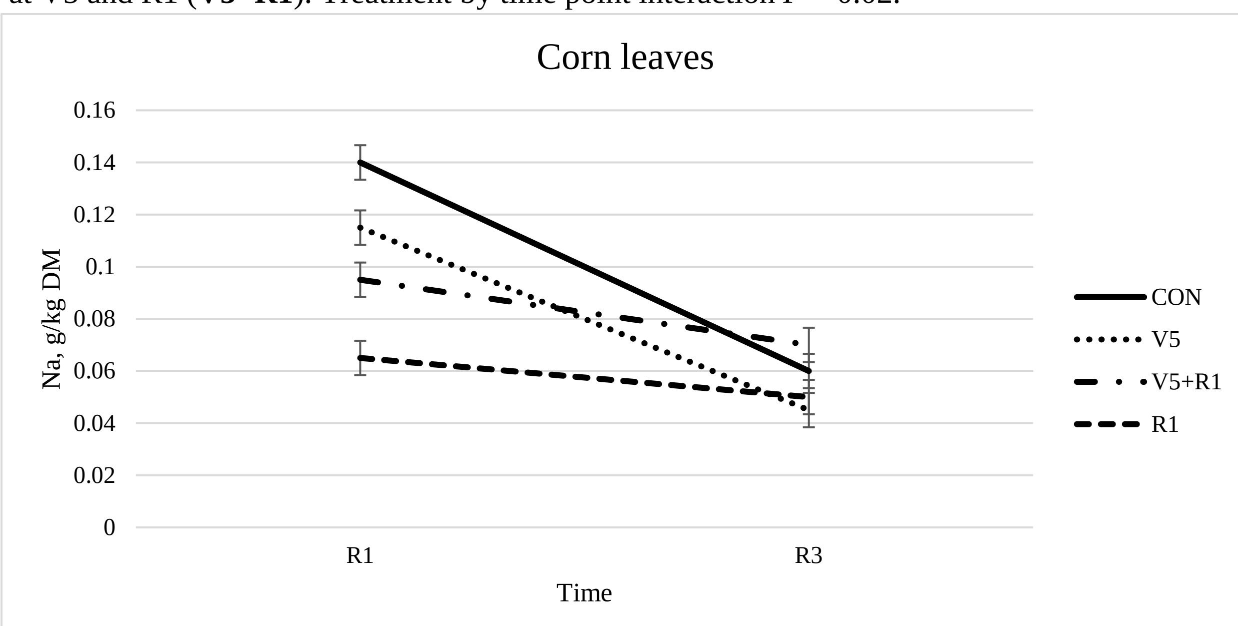


Figure 3.6. Zinc, ppm, for corn leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction P = 0.04.

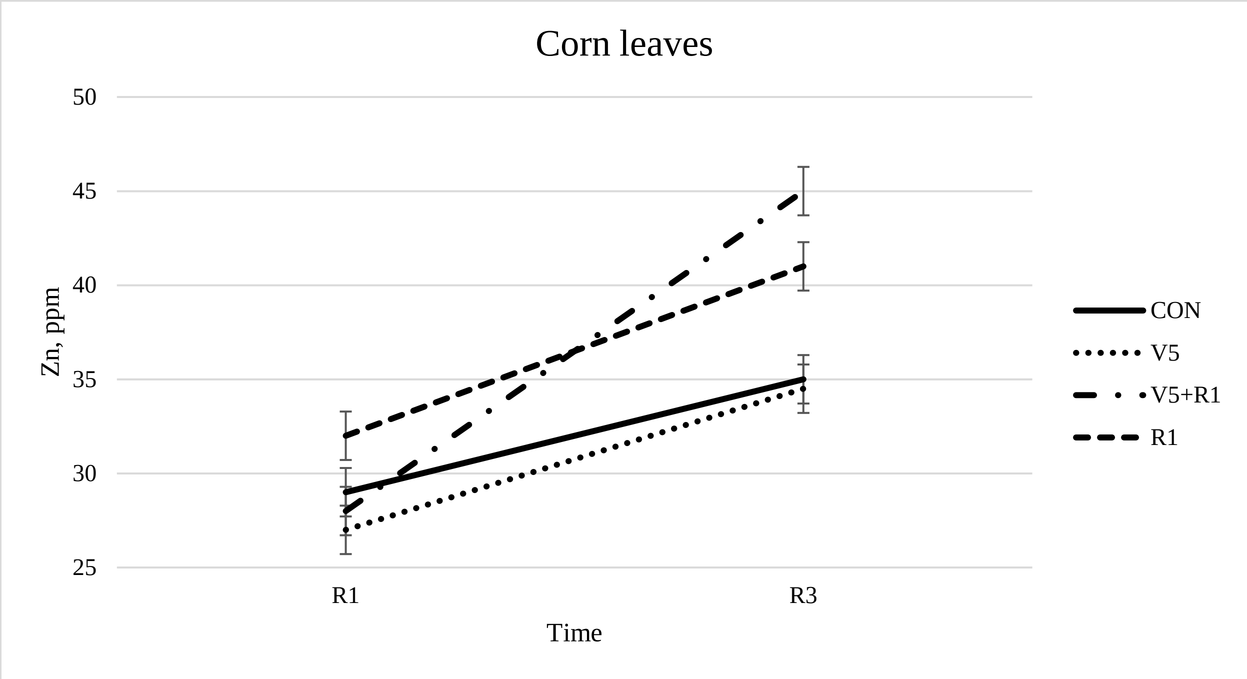


Figure 3.7. Copper, ppm, for corn leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction P = 0.02.

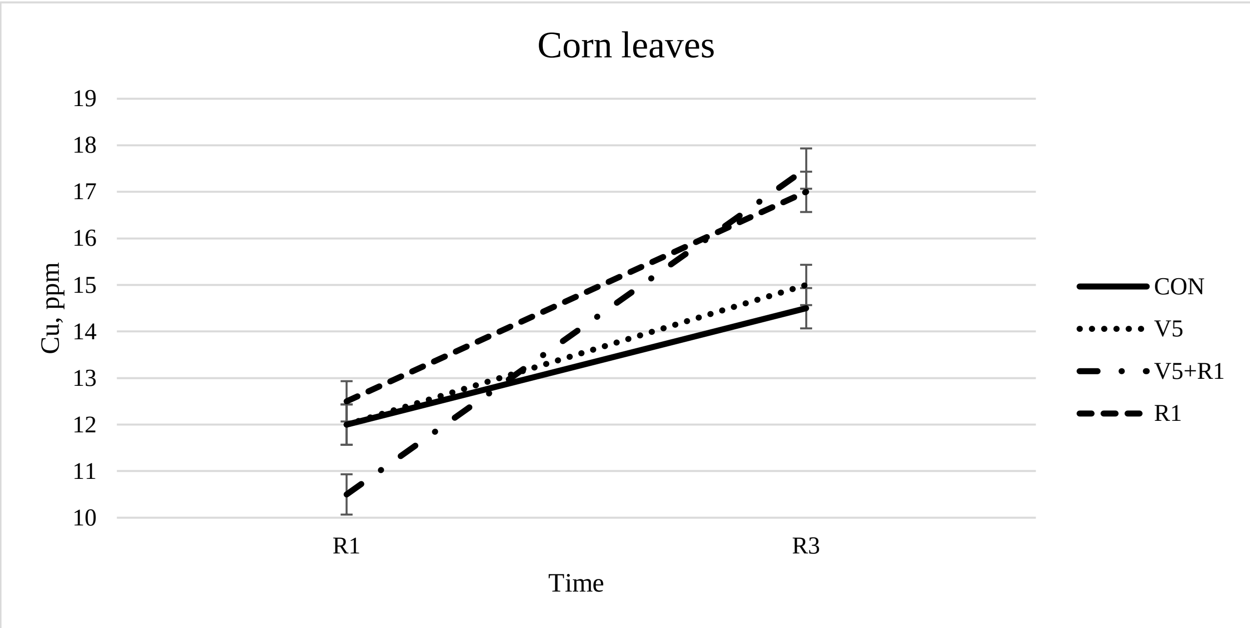


Figure 3.8. Cellulose, g/kg DM, for corn leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction $P = 0.007$.

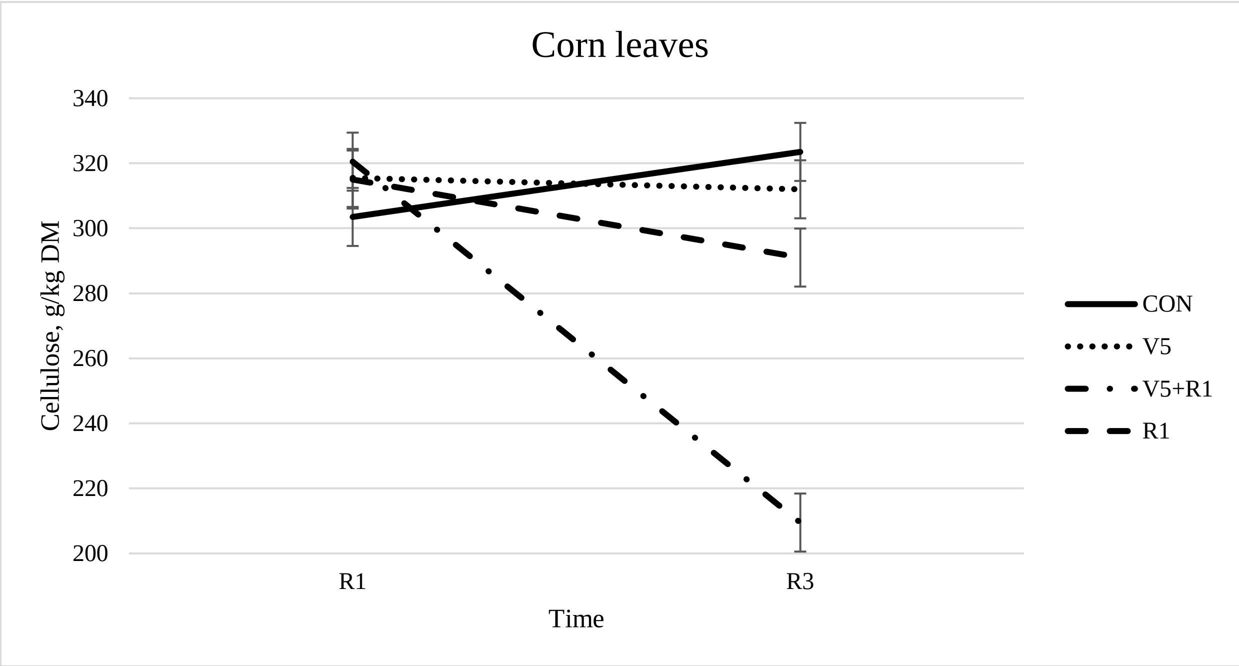
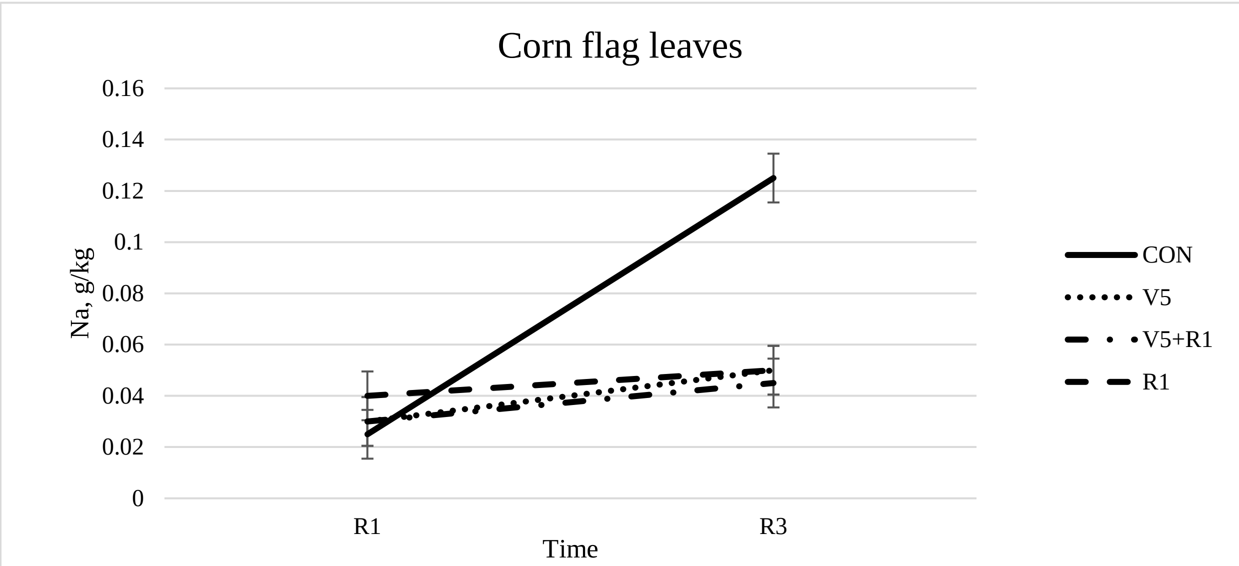


Figure 3.9. Sodium, g/kg DM, for corn flag leaves in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**). Treatment by time point interaction $P = 0.02$.



CHAPTER IV

Applications of foliar fungicide on corn for corn silage for ruminants II. Pyraclostrobin effects on corn silage composition

ABSTRACT

The objective of this study was to determine the effects of various applications of foliar fungicide on corn ensiled as corn silage. Treatments were replicated once and assigned to one of eight 0.4-ha plots of corn as follows: control (**CON**), corn receiving no foliar fungicide application; treatment 1 (**V5**), where corn received a mixture of pyraclotrobin and fluxapyroxad foliar fungicide (Priaxor, BASF Corp.) at corn vegetative stage 5 (V5); treatment 2 (**V5+R1**), where corn received two applications of foliar fungicide, a mixture of pyraclotrobin and fluxapyroxad at V5 and a mixture of pyraclostrobin + metconazole foliar fungicide (Headline AMP; BASF Corp.) at corn reproductive stage 1 (R1); treatment 3 (**R1**), in which corn received one application of pyraclostrobin + metconazole foliar fungicide at R1. Samples of corn silage were collected at harvest, prepared as 0.9-kg mini-silos and vacuumed sealed. Corn silage was ensiled for 0, 30, 90, and 150 d postharvest and frozen for later analysis. Treated corn silage had decreased dry matter (335, 319, 315, and 317 g/kg DM for CON, V5, V5+R1, and R1, respectively; $P = 0.0005$), but increased crude protein (81, 85, 82, and 87 g/kg DM for CON, V5, V5+R1, and R1, respectively; $P < 0.0001$), water soluble carbohydrates (38, 40, 46, and 52 g/kg DM for CON, V5, V5+R1, and R1, respectively; $P = 0.007$), and lactic acid (46.5, 50.1, 50.9, and 55.0 g/kg DM for CON, V5, V5+R1, and R1, respectively; $P = 0.0014$) compared with untreated corn silage. Corn silage in R1 had a lower lignin content (20 g/kg DM for R1 vs 24, 24, 26 g/kg DM for CON, V5, and V5+R1, respectively; $P = 0.03$), and corn silage in V5 had greater

milk kg/MT DM (1631 kg/ton DM for V5 vs. 1511, 1585, and 1576 kg/MT DM for CON, V5+R1, and R1, respectively; $P = 0.04$). Corn silage in R1 had a greater concentration of water soluble carbohydrates at 0 and 150 d postharvest (123 and 31.5 g/kg DM for 0 and 150 d, respectively; $P = 0.03$), and an increased lactic acid concentration at 90 d (71.1 g/kg DM for R1 vs. 63.4, 68.4, and 69.2 g/kg DM for CON, V5, and V5+R1, respectively; $P = 0.03$). Results suggest that fungicide application on corn at V5 or R1 may enhance the nutritive and fermentative profile for ruminants when ensiled of corn as corn silage.
Keywords: corn silage, foliar disease, fungicide, ruminant nutrition

INTRODUCTION

To feed meat and dairy producing ruminants yearlong, producers commonly store and preserve forage crops as silages at the time of harvest. The process of ensiling is broken down into four phases, each with varying lengths (Pahlow et al., 2003). Phase 1, the aerobic phase, is characterized by the reduction of atmospheric O_2 within a couple of hours postharvest; meanwhile, active proteases decompose proteins to amino acids and soluble carbohydrates. Phase 2, the fermentation phase, anaerobic microorganisms compete with one another for nutrients. For well-fermented silages, lactic acid bacteria (LAB) eventually dominate the microbial population and reduce the pH. Phase 2 can last anywhere from 1 wk to 1 mo (Pahlow et al., 2003). Phase 3, the stable phase, continues with the slow hydrolysis of structural and storage carbohydrates. When air is properly excluded from silage, theoretically, feedstuffs may last until needed for feed out. Phase 4, the feed out phase, is when silage is exposed to O_2 causing aerobic organisms to develop and decreasing the aerobic stability of the silage (Pahlow et al., 2003).

One of the most common silages fed to ruminants is corn silage. The USDA reported in 2014 that 89.4% of the United States dairy operations included corn silage in the diet of lactating cows (USDA, 2014). Under good forage management, silage from the previous harvest is enough to feed for the year until the new harvested crop has undergone all phases of ensiling. However, this is not always the case and producers need to feed the newly harvested silage as soon as possible. Length of ensiling has shown to have significant effects on DM (Der Bedrosian et al., 2012; Weinberg and Chen, 2013), lactic acid (Ferraretto et al., 2015) acetic acid (Der Bedrosian et al., 2012; Weinberg and Chen, 2013; Ferraretto et al., 2015), NDF digestibility (Der Bedrosian et al., 2012; Weinberg and Chen, 2013), and concentration of crude protein (Der Bedrosian et al., 2012).

On a DM basis, corn silage is included in the dairy diet at 40 to 60% of the total mixed ration. Dry matter intake and milk yield of cows decreased by the increasing the ADF, NDF, and lignin content of corn silage and decreasing the fiber digestibility (Oba and Allen, 2000). In a meta-analysis of 162 treatments, DMI and milk yield was 0.7 kg/d and 1.0 kg/d greater, respectively, for cows fed corn silage with high *in-vitro* NDF digestibility compared with a dual-purpose corn silage (Ferraretto and Shaver, 2015). Therefore, improvements in the nutritive quality and digestibility of corn silage may yield a greater lactation performance.

Unwanted fungal pathogens on corn may hinder the desired decreases in fiber and increases in digestibility of the plant content pursued by producers and nutritionists. Fungi attack plant cells and release toxins killing the plant tissue to provide nutrients for their growth (Sexton and Howlett, 2006). Lignification of the cell wall is a defense response of plants to both resist and defend against fungal enzymes (Vance et al., 1980). Physical damage to corn altered the NDF and ADF chemical composition of corn silage ensiled for 95 d (Teller et al., 2012). Furthermore, corn infected with Southern Rust resulted in increased NDF and ADF concentrations, and decreased *in-vitro* NDF digestibility when ensiled as corn silage (Queiroz et al., 2012). Moreover, corn plants inoculated with *Exserohilum turcicum*, the fungus causing Northern Leaf Blight, resulted in increased NDF and ADF concentrations when corn silage, but did not decrease true digestibility of NDF when fed to sheep (Wang et al., 2010).

Management practices, such as tillage and crop rotation, may not be enough to effectively manage fungal disease. Therefore, fungicide application may protect corn from fungi, limiting increases in fiber content and suffering corn yields. When fungal disease pressure in the field was severe, fungicide application on corn decreased the severity of diseased foliage compared with untreated corn (Bradley and Ames, 2010). A meta-analysis reported applications of pyraclostobin foliar fungicide on corn increased mean corn yield by 256 kg/ha (Paul et al., 2011). Moreover, applications of foliar fungicide are more likely to consistently increase yield and decrease stalk rot of corn plant, as well as, provide a positive economic return for producers when disease in the field is severe (Wise and Mueller, 2011).

Few studies have examined the effects of foliar fungicide on corn ensiled as corn silage and its effects on the nutritive and fermentation quality of corn silage for ruminants. Cows fed corn silage differing in foliar fungicide application showed a linear decrease in DMI as the number of applications increased, but constant milk production among treatments (Haerr et al., 2015). Cows fed corn silage treated with foliar fungicide tended to have better feed conversion values than those fed untreated corn silage (Haerr et al., 2015).

Therefore, the objective of this study was to determine the effects of foliar fungicide applications on corn at various times, then ensiled as corn silage for varying times post-harvest on the nutritive and fermentative quality of the feedstuff.

MATERIALS AND METHODS

Field preparation

Before winter 2014, manure was applied to the field where corn would be planted in the spring. Land was tilled conservatively using a Case IH Tiger Mate II (CNH Industrial, London, UK), making just one pass. Seven soil samples were collected from various places in the field and sent to a commercial laboratory (Rock River Lab, Watertown, WI.) for soil analysis. Soil samples were analyzed for pH, buffer pH, organic matter, phosphorus, potassium, calcium, magnesium, boron, manganese, zinc, and cation exchange capacity (CEC). Data for mean environmental temperature for Champaign-Urbana, IL and total rainfall were collected daily from planting until harvest from the state climatologist office for Illinois (Illinois State Water Survey, Prairie Research Institute, Champaign, IL).

Corn

The corn hybrid planted was Pioneer 1417AMXRR 2015 variety (Johnston, IA), the purpose of which is silage. Comparative relative maturity (CRM) for this hybrid is reached at 114 d. The hybrid of corn is marketed for having an outstanding silage yield, whole plant digestibility, and silage crude protein values. The variety is resistant to Gray Leaf Spot (caused by the disease *Cercospora zeae-maydis*) and Northern Leaf Blight (caused by the fungus *Exserohilum turcicum).* Corn seeds were planted on 30 April 20015 using a John Deere 7200 tractor (Moline, IL.). Eight 0.40-ha plots of corn were planted (40°04’58.8”N 88°13’08.4”W) at a planting density of 16,000 corn plants/ha.

Foliar fungicide application

Treatments were replicated once and randomly assigned to 1 of 8 0.4-ha plots of corn. Treatments were as follows: control (**CON**), corn receiving no foliar fungicide application; treatment 1 **(V5**), where corn received a mixture of pyraclotrobin ($C_{19}H_{18}ClN_3O_4$) and fluxapyroxad ($C_{18}H_{12}F_5N_3O$) (**PYR+FLUX**), foliar fungicide (Priaxor, BASF Corp.) at a rate of 0.15 kg of active ingredient (a.i.)/ha at corn vegetative stage 5 (V5) where the emergence of the fifth leaf is visible (Mueller and Pope, 2009); treatment 2 (**V5+R1**), where corn received two applications of foliar fungicide, a mixture of PYR+FLUX at 0.15 kg of a.i./ha at corn vegetative stage five, and a mixture of pyraclostrobin ($C_{19}H_{18}ClN_3O_4$) + metconazole ($C_{17}H_{22}ClN_3O$) foliar fungicide (**PYR+MET**; Headline AMP; BASF Corp.) at 0.15 kg of ai/ha at corn reproductive stage 1 (R1) or when the silks are fully extended (Mueller and Pope, 2009), treatment 3 (**R1**), in which corn received one applications of PYR+MET foliar fungicide at 0.15 kg of a.i./ha at corn reproductive stage 1.

Fungicide applications dates were 3 June (34 d post planting; corn growth stage V5), and 13 July 2015 (75 d post planting; corn growth stage R1). Applications of foliar fungicide were applied with a 4430 Case IH ground sprayer (CNH Industrial, London, UK) at 482 kPa of pressure with a 73-60-110 10 VS nozzle tip spraying at a volume of 168.54 L/ha. At each application, the sprayer was driven through all the treatments, even those not receiving fungicide to account for equal damage to the plant.

Disease evaluation

Two times during the growing season, corn was evaluated for foliar disease. Evaluations occurred on 11 July 2015, at corn reproductive stage 1 (R1) and on 13 August 2015, at reproductive phase 3 (R3) when kernels are yellow, with a milk white fluid (Mueller and Pope, 2009). Ten plants within each treatment were randomly selected for evaluation at each time point. Disease severity, as a percentage of leaf area, was estimated using three leaves: the ear leaf, one leaf above the ear leaf, and one leaf below the ear leaf; a method validated by Reis et al. (2007). The same evaluator walked through the treatments in the field and evaluated the plants at both time points to minimize error.

Harvest

Upon reaching the 3/4 milk stage of corn development, all treatments were harvested on 25 August 2015. Corn was chopped and processed using a New Holland FP240 forage chopper (CNH Industrial, London, United Kingdom). Theoretical length of chop was set to 1.9 cm and a kernel processor was used to improve digestibility of the corn kernels. At harvest, a minimum of three samples of chopped corn silage material from each treatment was composited to estimate dry matter. The DM for CON, V5, R1, and V5+R1 measured 26.5, 34.4, 27.7, and 33.2%, respectively. Chopped corn was transported by H&P forage wagons (H & S Manufacturing

Company Inc., Marshfield, WI) from field to scale (Mettler Toledo, Columbus, OH), where each full wagon was weighed and recorded; weights of wagons were not replicated.

Samples at Harvest

At harvest, a total of 5.5-kg of freshly cut corn was collected from three locations within the each plot in order to make silos. Treatments were composited in an identical fashion. A weighted 0.9-kg sample of corn silage was scooped and placed into a vacuum seal bag (28 cm × 36 cm). Using a vacuum sealer FoodSaver V845 Vacuum Packaging System (Food Saver, Boca Raton, FL.), four bags per replicated plot or 32 in total, were heat-sealed. To evaluate the effect of foliar fungicide on corn silage through time, 1 of the 4 treatment silos was labeled and removed at 1 of 4 times postharvest: 0 d (25 August 2015), 30 d (24 September 2015), 90 d (23 November 2015), and 150 d (21 January 2016). All silos were stored in a dark laboratory room, with an average temperature of 21°C. On 0 d, a total of 8 silos were removed and frozen at -20°C for later nutrient analysis. On the remaining time points (d 30, 90, and 150), 8 silos in total were removed each time and frozen at -20°C for later nutrient analysis.

Nutrient Analysis

Once all corn silage silos were collected and stored at -20°C for a minimum of 1 wk, bags were opened and composited in an identical fashion. One representative sample of corn silage from each plot at all times (n = 32) was sent for laboratory analysis. All samples were analyzed for dietary DM, crude protein, soluble protein, NDF, ADF, fat, lignin, starch, and ash using wet chemistry at a commercial laboratory (Dairy One, http://dairyone.com/wp-content/uploads/2014/02/Forage-Lab-Analytical-Procedures-Listing-Alphabetical-July-2015.pdf, 2015a).

Briefly, corn silage samples were dried in force air oven at 60°C (Goering and Van Soest, 1970). For analysis of ADF, 0.5-g samples were digested for 75 min as a group of 24 in 2L of ADF solution in ANKOM A200 digestion unit. Samples were rinsed 3 times with boiling water for 5 min in filtered bags and then soaked for 3 min in acetone, followed by drying at 105°C for 2 h (AOAC International, 2000; ANKOM, 2011). For an analysis of lignin, samples were subject to same treatment as for ADF analysis, and residue was digested as a group of 24 with 72% w/w sulfuric acid for 3 h in ANKOM Daisy incubator (AOAC International, 2000; ANKOM, 2011). For an analysis of NDF, 0.5-g samples in filter bags were digested for 75 min as a group of 24 in 2 L of NDF solution in ANKOM A200 digestion unit. Four milliliters of alpha amylase and 20g sodium sulfite were added at the start of digestion. Samples were rinsed 3 times with boiling water for 5 min, and alpha amylase was added in the first 2 rinses. After rinses, bags are soaked for 3 min in acetone, followed by drying at 105°C for 2 h (Van Soest et al., 1991; ANKOM, 2011;). Using the NRC (2001) equation for total digestible nutrients (**TDN**) and net energy for lactation (**NE$_L$**) were calculated.

A fermentation profile was determined, including pH, percentage of lactic acid, percentage of acetic acid, percentage of propionic acid, percentage of butyric acid, ammonium-N of total nitrogen, percentage of total acid, VFA score, *in vitro* true digestibility 30-h (IVTD 30h), NDF digestibility 30-h (NDFD 30h). For preparation of samples for volatile fatty acid analysis, 50-g samples of corn silage were blended at 20000 rpm for 2 min in 750 mL of deionized water, filtered through cheesecloth, and filtered again with a disposable syringe filter. Briefly, acetic, propionic, butyric, and iso-butyric acid were analyzed using gas chromatography, using 100 ppm trimethylacetic acid and a Perkin Elmer Autosystem XL Gas Chromatograph. Lactic acid for corn silage samples was analyzed using YSI 2700 SELECT Biochemistry analyzer with a L-

Lactate membrane. Neutral detergent fiber digestibility 30-h was determined by incubating dry, ground samples in a buffer/rumen fluid mixture as described by Goering and Van Soest (1970) for 30-h, under anaerobic conditions at 39°C. Samples were then subject to fiber analysis as previously described within this article. A VFA score was developed by the commercial laboratory to assist producers and advisors. The score weighs the positive impact of lactic and acetic acid with the negative impact of butyric acid to arrive at one score; a score of 8 to 10 indicates a good quality silage and less than 3 indicated a poor silage (Dairy One, 2015b). Lastly, corn silage samples were subject to corn processing score and milk kg/ ton DM (Dairy One, 2015a). Corn processing score, also referred to as kernel processing score, is calculated by the subtracting the percentage of starch that did not pass through the 4.75 mm sieve from total percentage of starch (Ferreira and Mertens, 2005).

Additionally, corn silages from 0 and 150 d were analyzed for mycotoxins including: aflatoxin B1, aflatoxin B2, aflatoxin B3, aflatoxin G1, aflatoxin G2, 3-acetyl deoxynivalenol (DON), 15-acetyl DON, vomitoxin, T-2, and zearalenone at a commercial lab (Dairy One, New York). Aflatoxin B1, aflatoxin B2, aflatoxin B3, aflatoxin G1, and aflatoxin G2 was determined using AOAC 994.08 (AOAC International, 2005). The 3-acetyl DON, 15-acetyl DON and vomitoxin contents were determined using analytical procedures described by Trucksess et al. (1997) and MacDonald et al. (2005b). Mycotoxin T-2 was determined using an analytical procedure as described by Croteau et al. (1994). Zearalenone was determined using an analytical procedure as described by MacDonald et al. (2005a). In brief, the mycotoxin sample is extracted from the corn using an acetonitrile/water (80/20) extraction method. Extracted mycotoxins samples are then prepared as solid phase extracts to using a Triology MT3000 clean up column

(Trilogy Analytical Laboratory, Missouri) and analyzed using a liquid chromatography-mass spectrometry technique.

Statistical analysis

Using SAS (v. 9.4, S.A.S Institute Inc., Cary NC.), data was statically analyzed using a completely randomized design. Treatment means collected at 0, 30, 90, and 150 d postharvest were used to make inferences about the nutrient analysis results and fermentation analysis results. Data were analyzed using the MIXED procedure of SAS by the following model:

$$Y_{ijk} = \mu + F_i + T_j + F_i \times T_j + R_k + e_{ijk},$$

where F_i = the effect of foliar fungicide treatment, T_j = the time effect of days postharvest ensiled, F_i x T_j = the effect of the interaction between foliar fungicide i and time effect j, R_k = the replicate effect of k, and e_{ijk} is the random residual error. The model included fixed effects of treatment and time point, with random effect for replication and replication by treatment. The degrees of freedom method was Kenward-Rogers (Littell et al., 1998). Results are reported as least squares (LSM) means with corresponding standard errors of the mean (SEM) for fixed effects of foliar fungicide treatment. Least squares means with corresponding SEM for only significant fixed effects of time ensiled postharvest are included in this manuscript; other values were not reported. Ammonia as a percentage of soluble protein, and sulfur concentration were log transformed for better distribution of values and variance of residuals. The log transformed data was back transformed and presented as LSM and SEM in tables. Significant interactions between fixed effects are presented as figures. Residuals distribution was evaluated for normality and homoscedasticity. Statistical significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq$

0.10. Results for non-replicated data reported as means with corresponding standard deviation (SD).

RESULTS

Corn yield and environmental results

The total yield for all treatments averaged 81.3 x 10^3 kg/ha. Corn yield in CON, V5, V5+R1, and R1 totaled 78.0, 83.0, 81.3, and 82.9 x 10^3 kg/ha, respectively. During the growing season, the average daily temperature was 21.6 ± 7°C. Total rainfall in Champaign-Urbana, IL was 218.4 cm.

Disease evaluation

Foliar diseases including common rust, Northern Leaf Blight, and Gray Leaf Spot was present at the R1 evaluation and the R3 evaluation. At R1, common rust was not seen on corn in CON, corn in V5, or corn in R1, and only 1% of leaf area (**LA**) of corn in V5+R1; Northern Leaf Blight was not seen on corn in V5, but was 1% of LA of corn in V5+R1, and 3% of LA of corn in CON and corn in R1; and Gray Leaf Spot was seen in on corn in V5, corn in V5+R1, and corn in R1 at 1% of LA, and 2% of LA on corn in CON. At R3, common rust was not seen on corn in CON or any treatment of foliar fungicide; Northern Leaf Blight was 2% of LA of corn in V5+R1 and corn in R1, 6% of LA of corn in V5, and 10% LA of corn in CON; Gray leaf spot was not seen on corn in R1, but was seen at 1% of LA of corn in V5+R1, 9% of LA of corn in CON, and 15% of LA of corn in V5.

Corn silage nutrient and fermentation analysis as an effect of treatment

Nutrient analysis results of corn silage from corn in CON, V5, V5+R1, and R1 due to the fixed effects of treatment are in Table 4.1.

Corn silage composition

Corn silage in V5, V5+R1, and R1 had less DM when compared with corn silage in CON ($P = 0.0005$). Corn silage in R1 had the greatest CP when compared with corn silage in CON, V5, and V5+R1. Corn silage in R1 had a lower concentration of lignin when compared with corn silage in CON, V5, V5+R1 ($P = 0.03$). Corn silage in V5+R1 and in R1 had a greater concentration of WSC when compared with corn silage in CON and V5 ($P = 0.007$). Corn silage in R1 had greater concentration of ash when compared with corn silage in CON, V5, and V5+R1 ($P = 0.0045$). Corn silage in R1 had greater concentration of phosphorus when compared with corn silage in CON, V5, and V5+R1 ($P < 0.0001$). Corn silage in R1 had a greater concentration of potassium when compared with corn silage in CON, V5, and V5+R1 ($P < 0.0001$). Corn silage in R1 had a greater concentration of sulfur when compared with corn silage in CON, V5, and V5+R1 ($P = 0.03$). Corn silage in CON had greater concentration of sodium when compared with corn silage in V5, V5+R1, and R1 ($P = 0.002$). Corn silage in CON had a greater concentration of iron when compared with corn silage in V5, V5+R1, and R1 ($P < 0.0001$). Corn silage in V5+R1 had a lower concentration of manganese when compared with corn silage in CON, V5, and R1 ($P < 0.0001$).

Corn silage energy calculations

Results are also in Table 4.1. No differences due to the effect of foliar fungicide treatment were observed for the NE_l, NE_g, NE_m, or TDN, per kilogram DM for corn silage in CON, V5, V5+R1, and R1.

Corn silage fermentation products

Fermentation analysis results of corn silage from corn in CON, V5, V5+R1, and R1 due to the fixed effects of treatment are in Table 4.1. No differences were observed in pH for corn silage from CON, V5, V5+R1, and R1. Corn silage in R1 had a greater concentration of lactic acid when compared with corn silage in CON, V5, and V5+R1 (P = 0.0014). Corn silage in R1 had a greater concentration of total acid when compared with corn silage in CON, V5, and V5+R1 (P = 0.0002). Corn silage in V5, V5+R1, and R1 had greater volatile fatty acid (VFA) scores when compared with corn silage in CON (P = 0.01). Corn silage in V5 had a greater milk kg/ MT DM when compared with corn silage in CON (P = 0.04). Corn silage in V5+R1 had a lower kernel processing score when compared with corn silage in V5 (P = 0.01).

Corn silage mycotoxins

Mycotoxin analysis from corn silage in CON, V5, V5+R1, and R1 due to the fixed effects of treatment is in Table 4.1. Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, 3-acetyl DON, 15-acetyl DON, and T-2 were not detected for corn silage in any treatment. Zearalenone was only detected in one sample from corn silage in R1 at a concentration of 1.4 ppm. Corn silage in R1 had a greater concentration of vomitoxin detected when compared with corn silage in CON, V5, and V5+R1 (P = 0.004).

Corn silage nutrient and fermentation analysis as an effect of time postharvest

Nutrient analysis results of corn silage from corn in CON, V5, V5+R1, and R1 due fixed effects of time postharvest are in Table 4.2. Only significant and non-interaction values are presented.

Corn silage composition

Dry matter decreased in corn silage samples ensiled for 0, 30, 90, and 150 d postharvest (P = 0.03). Crude protein concentration increased in corn silage samples ensiled for 0, 30, 90, and 150 d postharvest (P < 0.0001).

Corn silage fermentation profile

All results are also in Table 4.2. Corn silage samples ensiled for 30, 90, and 150 d postharvest resulted in a lower pH than corn silage ensiled for 0 d postharvest ($P < 0.0001$). Acetic acid concentration increased for corn silage samples ensiled for 0, 30, 90, and 150 d postharvest ($P < 0.0001$). Ammonium-N, as a percentage of nitrogen, increased for corn silage samples ensiled for 0, 30, 90, and 150 d postharvest ($P < 0.0001$). Volatile fatty acid score increased for corn silage samples ensiled for 0, 30, 90, and 150 d postharvest ($P < 0.0001$).

Foliar fungicide treatment by time postharvest interaction

Significant foliar fungicide treatment by ensiling time postharvest interactions were observed in corn silage samples for lignin (Figure 4.1), WSC (Figure 4.2), Na (Figure 4.3), Fe (Figure 4.4), lactic acid (Figure 4.5), total acid (Figure 4.6), ammonia (Figure 4.7), and milk kg/ MT DM (Figure 4.8). Corn silage in R1 resulted in a lower concentration of lignin ensiled for 90 d when compared with corn silage in CON, V5, and V5+R1 ($P = 0.04$). Corn silage in all treatments rapidly declined in the concentration of WSC as the number of days ensiled postharvest increased ($P = 0.03$). Corn silage in CON had a greater concentration of Na at 0 d, when compared with corn silage in V5, V5+R1, and R1, but as the days ensiled postharvest increased to 150 d, no differences were observed ($P < 0.0001$). Corn silage in CON had a greater concentration of Fe at 0 and 30 d postharvest when compared with corn silage in V5, V5+R1, and R1 ($P = 0.03$). Corn silage in R1 had a greater concentration of lactic acid at 30 d postharvest when compared with corn silage in CON ($P = 0.03$). Corn silage in R1 had greater concentration of total acid 30, 90, and 150 d postharvest when compared with corn silage in CON ($P = 0.005$). Corn silage in CON had greater concentration of ammonia at 0 d, when compared with corn silage in V5, V5+R1, and R1 ($P = 0.02$). Corn silage in R1 had a greater projection of milk kg/MT DM at 90 d postharvest, when compared with corn silage in CON, V5, and V5+R1 ($P = 0.04$).

DISCUSSION

The aim of this study was to determine the effects of applications of foliar fungicide on corn at different developmental growth stages, and how application timing may impact the nutritive and fermentative quality once ensiled as corn silage for varying times postharvest. We hypothesized that fungicide application on corn at R1 may positively affect the nutritive and fermentation profile the most compared to other application timings.

Overall, the purpose of foliar fungicide application on corn is to limit the negative effects of fungal pathogens on the plant material. At the R1 evaluation, foliage in CON was infected the most with disease, constituting 5% of total leaf area. According to recommendations of Paul et al. (2011), when greater than 5% of the LA is diseased, decisions to use fungicide may be more warranted and cost effective. At the R3 evaluation, total diseased tissue accounted for 19% of the total LA in CON, and 21% of the total LA in V5. In this study, 35 d elapsed between the evaluation at R1, and the evaluation at R3. Above normal rain during the summer of 2015 and cooler June and July temperatures were the ideal conditions for the growth and development of fungi. Even though fungicide was applied at V5, it was 61 d before the evaluation of disease at R3; active ingredients within fungicide only remain in the waxy cuticle for an average of 21 days post application (Balba, 2007). Fungicide application at R1 may provide greater fungal protection during the crucial reproductive stages of grain fill than earlier applications of fungicide.

Dry matter content was lower for corn silage with applications of foliar fungicide when compared with CON (Table 4.1). Disease in field may be the cause of increased DM content in CON. Queiroz et al. (2012) reported that increasing Southern Rust infection on growing corn, increased the DM content of corn silage once ensiled compared with lower concentrations of infection. On the other hand, Wang et al. (2010) inoculated corn with Northern Leaf Blight, and reported a decreased DM content of corn silage once ensiled when compared with not inoculated. In the current study had disease been the cause of increased DM content in CON, then DM content in V5 would have been expected to be increased, as it too was heavily diseased in the field. Foliar fungicide application on corn can also cause delays in the senescence of leaves and increase the amount of time needed for the crop to dry down (Wise and Mueller, 2011). In the current study, fungicide application on the corn ensiled as corn silage may have delayed the senescence of the leaves and stalk as the plant aged. Additionally, DM content varied with time postharvest, with a general decreasing trend as the number of days increased (Table 4.2). Herrmann et al. (2011) reported similar results, as ensiling corn silage for 365-d compared to fresh silage decreased DM content 2.5%. Ferraretto et al. (2015) reported that DM concentration was unaffected by length of ensiling when evaluating the effects of prolonged storage on corn silage. Der Bedrosian et al. (2012) found a general tendency for increased DM with increasing length of storage.

Acid detergent fiber content was unaffected by both foliar fungicide treatment (Table 4.1) and length of ensiling (Table 4.2). Wang et al. (2010) found concentrations of ADF in corn silage to be unaffected when inoculating corn with Northern Leaf Blight. However, Queiroz et al. (2012) reported that ADF concentration of corn silage to increase linearly as rust infection on the plant increased. In the study of Der Bedrosian et al. (2012), length of ensiling did not have an effect on the ADF concentration, matching our results. Similarly, no difference for NDF concentration in treatment corn silage was observed (Table 4.1). Wang et al. (2010) reported NDF concentration to be unchanged in corn silage when inoculated with disease, but data from Queiroz et al. (2012) showed NDF concentration to increase linearly as Southern Rust on the plant increased. Variability between studies may be the result of different environmental conditions during the growing season, soil fertility, and amount of disease on the plant. No differences in the concentrations of NDF were observed as time postharvest increased (Table 4.2). Others have suggested similar results of NDF concentration relative to ensiling time (Der Bedrosian et al., 2012; Ferraretto et al., 2015). Corn silage in R1 resulted in 4 and 6 g/kg DM less lignin content when compared with corn silage in CON and V5+R1, respectively (Table 4.1). Foliar fungicide applications on corn have shown to improve standability and reduce stalk lodging (Wise and Mueller, 2011). Therefore, the increased lignin concentration in V5+R1 may be the result of improving stalk health. The increase in lignin concentration of corn silage in CON and V5 relative to corn silage in R1 may be result of the disease differences as evident by the percentage of diseased foliage. Additional lignification of the secondary cell wall can result when the plant senses a pathogen and triggers the need for a barrier to prevent further pathogen invasion by either enzymes or turgor pressure. A significant foliar fungicide treatment by ensiling length postharvest was observed for lignin concentration of corn silage (Figure 4.1). Corn silage in R1 resulted in the lowest concentration of lignin reporting 12 g/kg DM, when ensiled for 90 d, but then increased to 24 g/kg DM when ensiled for 150 d. A similar trend was observed for acid detergent lignin when ensiled for different lengths; where acid detergent lignin decreased 0.8% from 90 to 180 d, but then increased 0.7% from 180 to 365 d (Herrmann et al., 2011). However, Der Bedrosian et al. (2012) found no difference for the concentration of lignin due to the effect of prolonged ensiling time. Applications of foliar fungicide on corn ensiled as corn silage numerically increased NDF digestibility 30-h by 13, 23, and 35 g/kg DM for V5, V5+R1, and R1, respectively, when compared with CON (Table 4.1). Queiroz et al. (2012) reported increased infection significantly decreased the NDF digestibility 48-h of corn silage compared to less infected corn silage. Application of fungicide at R1 resulted in the most digestible feedstuff, which may be the result of proper application timing relative to the increased foliar disease between the R1 and R3 evaluations. In a meta-analysis, a one unit increase in *in vitro* or *in situ* NDF digestibility of corn silage was associated with a 0.25 kg/d increase in 4% FCM yield (Oba and Allen, 1999). Furthermore, in an analysis of 96 treatments, an increase of 0.01 in NDF organic matter digestibility of corn silage increased DMI 0.02 kg and milk yield 0.08 kg (Krämer-Schmid et al., 2016). Differences in digestibility were not observed for corn silage ensiled for varying times postharvest. One study reported a decrease in digestibility within the first 45 d, but then no difference in digestibility from 45 to 365 d (Der Bedrosian et al., 2012). Another study found a continued decreased in NDF digestibility 30-h ensiled for up to 6 mo (Weinberg and Chen, 2013). Variability could be due to differences in techniques used, but also differences in sample sizes, as decreasing the sample size increased the digestibility of NDF (Malebana et al., 2015).

Starch concentration was unaffected by foliar fungicide application and time postharvest ensiled (Table 4.1). Had the corn ears been damaged from either insects or hail, then it may have been expected for the starch content of corn silage to decrease compared with undamaged corn silage (Teller et al., 2012). Some have reported no change in starch concentration of corn silage when ensiled for an increasing amount of days (Der Bedrosian et al., 2012; Ferraretto et al., 2015). Concentration of WSC increased for corn silage in V5+R1 and R1 when compared to

CON (Table 4.1). Applications of foliar fungicide on corn at R1, or tassel, have shown to have higher grain yields when compared to earlier timings of application (Testa et al., 2015). Because fungicide application at R1 happened during a crucial time of grain fill, it may have positively affected the WSC concentration of the corn silage the most compared to application at V5. A significant interaction between foliar fungicide treatment and ensiling time for corn silage reflects the use of WSC by microorganisms during the aerobic phase (Figure 4.2). Initial differences in the concentration of WSC appear at 0 d for all treatments, but small differences once the anaerobic period begins (Figure 4.2).

Among treatments, pH was not statistically different, but numerically corn silage in V5+R1 had 0.1 unit decrease in pH when compared with corn silage in CON (Table 4.1). Furthermore, corn silage in V5 and corn silage in R1 had 0.02 and 0.03 lower pH than CON (Table 4.1). Again, this could be an indicator of the presence of disease in the field. One study reported that increasing the amount of infection on corn ensiled as corn silage resulted in a greater pH than control (Quieroz et al., 2012). In the current study, pH of fresh samples measured 5.7, but decreased early in the ensiling process stabilizing before 30 d to 3.8 (Table 4.2). Others have described similar trends in the stabilization of pH (Der Bedrosian et al., 2012; Ferraretto et al., 2015). Acetic acid concentrations were not different by treatment (Table 4.1), but increased with storage time (Table 4.2). Storing corn silage for extended periods of time resulted in a greater accumulation of acetic acid concentration as the number of days increased (Der Bedrosian et al., 2012; Weinberg and Chen, 2013; Ferraretto et al., 2015). Lactic acid concentration was greatest for corn silage in R1, 8.5 units above corn silage in CON (Table 4.1). Queiroz et al. (2012) increased the concentration of disease on corn, then ensiled it as corn silage, reporting a worse fermentation profile, denoted by decreased lactic acid concentration, for corn silage from diseased corn. Applications of foliar fungicide on diseased-pressured corn may increase the fermentation quality of the corn silage. A significant interaction between foliar fungicide application on corn and ensiling length may indicate fungicide application reduces the time required to depletion of O_2 in the aerobic phase and quicker entry into the anaerobic phase of ensiling. This is further supported by the increased concentration of WSC in corn silage treated with fungicide when compared to control (Figure 4.2). By creating a better fermentation environment, fungicide application on corn ensiled as corn silage may have allowed aerobic bacteria less time to metabolize substrates such as WSC, resulting in the increased concentration of both WSC and lactic acid sooner in the ensiling process. Additionally, corn silage in R1 resulted in the greatest concentration of lactic acid at 30 d (Figure 4.5). Increased lactic acid concentration after 30 d may be beneficial for producers who need access to feed corn silage as soon as possible.

Milk kg/ MT of DM for corn silage in V5 projected an increase of 120 milk kg/ MT of DM when compared to CON (Table 4.1). Lower NDF and ADF concentrations, and greater NDF digestibility may be the reason for the increased milk projection for corn silage in V5 (Table 4.1). Ivan et al. (2005) reported an increase of 2.2 kg of milk, and a difference of 4.1 units *in vitro* NDF digestibility-30 h for highly digestible corn silage fed to cows when compared with less digestible corn silage. The authors hypothesized the increase in milk production was a function of increased digestibility, allowing a greater DMI. At 90 d, treated corn silage projected a greater yield of milk kg/ MT of DM (Figure 4.5). In a laboratory study, not fed to cows, corn silage treated with foliar fungicide projected an increase of 37 kg milk/ MT of DM when compared to untreated, suggesting a 1.8 percentage unit increase in NDF digestibility may be the cause (Blonde and Esker, 2008).

Kernel processing score was 8.95 units greater for corn silage in V5 compared with corn silage in V5+R1 (Table 4.1). Kernel processing score is used to assess how well a kernel has been mechanically processed at harvest and has been associated with differences in starch utilization for ruminants. The increase in kernel processing score for corn silage in V5 may be due to an increased concentration of starch, but also a softer kernel endosperm to fragment. Softer kernels are easier to fragment and allow greater amount of starch to pass through the sieve. Data from Ferreira and Mertens (2005) showed *in vitro* DM disappearance was greater for ground kernels in corn silage when compared to whole kernels in corn silage. This led us to hypothesize that corn kernels from corn silage in V5+R1 may not be as digestible for ruminants as corn kernels from corn silage in V5. The same study used a negative regression to reveal that most starch in unprocessed kernels and minimally fragmented kernel pieces do not ferment *in vitro* (Ferreira and Mertens, 2005). Testa et al. (2015) performed a floating test, used to assess the density of corn kernels, and a total milling energy test, used to quantify the amount of power needed to mill the corn kernel, on kernels from corn previously sprayed with foliar fungicide. Testa et al. (2015) reported fungicide application at R1 increased kernel hardness evident in a higher milling energy and lower density using the floating test, while applications of fungicide at mid stem elongation resulted in kernels with a higher density (Testa et al., 2015). More research is needed, but application of fungicide on corn at R1 may require corn kernels to be greater processed when harvested for corn silage, allowing greater digestibility of starch by the cow.

CONCLUSIONS

Applications of fungicide on corn, later ensiled as corn silage resulted in less DM content compared with untreated corn silage. Corn silage from corn with fungicide treatment at R1 resulted in the lowest concentration of lignin, and greatest concentration of lactic acid. Corn silage in V5 projected the most milk kg / MT of DM when compared to CON. As ensiling time postharvest increased, DM content decreased and pH decreased. Significant interactions between fungicide application and number of days ensiled postharvest occurred for lactic acid concentration, milk kg/ MT of DM, WSC content, and lignin concentration. Applications of fungicide at V5 or R1 may reduce the effects of fungal disease on corn plants ensiled as corn silage, enhancing their nutritive and fermentative profile when fed to ruminants.

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TABLES AND FIGURES

Table 4.1. Least squares means and associated standard errors for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**) or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatments[1]					*P*-value
						Fixed effects[2]
	CON	**V5**	**V5+R1**	**R1**	**SEM**	**Trt**
Corn Silage Composition						
DM, g/kg	335[a]	319[b]	315[b]	317[b]	2.6	0.0005
CP, g/kg DM	81[c]	85[b]	82[c]	87[a]	0.5	<0.0001
Soluble P, g/kg CP	516	509	519	508	7.3	0.65
Ammonia, % Soluble P[3]	144	79	76	79	28.2	0.07
ADF, g/kg DM	250	238	257	247	6.8	0.29
NDF, g/kg	412	389	418	401	9.8	0.23
Lignin, g/kg DM	24[abc]	24[ab]	26[a]	20[d]	1.0	0.03
NFC, g/kg DM	429	449	420	428	10.3	0.50
Starch, g/kg DM	305	338	305	311	12.6	0.25
WSC, g/kg DM	38[b]	40[b]	46[a]	52[a]	2.5	0.007
Crude Fat, g/kg DM	29	33	30	29	0.9	0.06
Ash, g/kg DM	49[b]	51[b]	51[b]	54[a]	0.8	0.0045
Ca, g/kg DM	2.3	2.2	2.2	2.1	0.07	0.20
P, g/kg DM	2.9[c]	3.1[b]	3.0[b]	3.2[a]	0.02	<0.0001
Mg, g/kg DM	1.7	1.6	1.7	1.5	0.06	0.35
K, g/kg DM	10.7[c]	10.8[c]	11.6[b]	12.4[a]	0.2	<0.0001
S, g/kg DM	1.2[b]	1.2[ab]	1.2[b]	1.3[a]	0.02	0.03
Na, g/kg DM	0.04[a]	0.03[b]	0.02[c]	0.02[c]	0.003	0.002
Fe, ppm	105.3[a]	88.1[c]	89.1[c]	96.13[b]	1.67	<0.0001
Zn, ppm	23.8	25.9	25.0	26.0	0.65	0.10
Cu, ppm	6.1	6.1	5.6	6.1	0.19	0.22
Mn, ppm	15.5[a]	15.0[ab]	12.8[c]	14.8[ab]	0.26	<0.0001
Molydbenum, ppm	0.7	0.6	0.7	0.6	0.06	0.54
Energy Calculations[5]						
NE_l, MJ/kg	6.58	6.80	6.64	6.76	0.08	0.31
NE_g, MJ/kg	4.02	4.24	4.08	4.52	0.18	0.27
NE_M, MJ/kg	6.74	6.79	6.62	6.72	0.11	0.41
TDN	689	704	695	703	0.72	0.45
Fermentation Products						
pH	4.31	4.29	4.21	4.28	0.03	0.23
Ammonia	3.7	3.7	3.4	3.7	0.07	0.08
Lactic Acid, g/kg	46.5[c]	50.1[b]	50.9[b]	55.0[a]	1.1	0.0014
Acetic Acid, g/kg	9.8	8.9	9.0	9.3	0.5	0.62
Lactic/acetic ratio	3.96	4.46	4.43	4.73	0.23	0.17
Total acid, g/kg	56.3[c]	59.0[b]	59.5[b]	64.3[a]	0.8	0.0002
Amm-N, g/kg total N	45	43	40	41	1.4	0.13
VFA score	6.80[c]	7.00[ab]	7.01[ab]	7.17[a]	0.07	0.01
IVTD 30 h, g/kg DM	783	796	789	800	7.0	0.34
NDFD 30 h, g/kg DM	470	483	493	505	11.9	0.25
Kd, % per hour	0.03	0.03	0.03	0.03	0.001	0.48
Milk kg/MT DM	1511[b]	1631[a]	1585[ab]	1576[ab]	25.8	0.04

(Table 4.1 continued)

Milk kg/proc MT DM	1539	1646	1585	1579	30.1	0.15
Kernel Processing Score[6]	66.64[ab]	68.40[a]	59.45[c]	64.25[abc]	1.68	0.01
Mycotoxins						
Vomitoxin, ppm	1.18[b]	1.05[b]	1.05[b]	2.05[a]	0.09	0.004

[1] Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application at V5), R1 (with 1 application at R1), and V5+R1 (with 2 applications at V5 and R1).
[2] Fixed effects of (TRT) effect due to fungicide treatment on corn plants with superscripts denoting statistical differences between treatments.
[3] Log transformation *P*-values presented, non-transformed data presented.
[4] Water soluble carbohydrates.
[5] NRC (2001).
[6] Total starch in the sample - the percentage of starch that did not pass through the 4.75 mm sieve = the kernel processing score.

Table 4.2. Least squares means and associated standard errors for corn silage ensiled for 0 days, 30 days, 90 days, or 150 days postharvest for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**) or two applications of foliar fungicide at V5 and R1 (**V5+R1**).

	Treatments[1]																	*P*-value
	CON				V5				V5+R1				R1				SEM	Fixed Effect[2]
	0	30	90	150	0	30	90	150	0	30	90	150	0	30	90	150		TP
Composition																		
DM, g/kg	351	320	345	326	323	312	315	326	315	313	314	319	318	313	327	310	5.3	0.03
CP, g/kg DM	77	84	82	83	82	86	87	87	79	83	83	83	84	88	87	91	0.9	<0.0001
SP, g/kg CP[3] DM	400	525	575	565	395	500	570	570	410	490	595	580	415	500	540	575	14.7	<0.0001
Ammonia, g/kg SP[4]	60	75	100	340	40	80	95	100	35	80	85	105	35	85	95	100	56.4	<0.0001
Ash, g/kg DM	48	51	47	52	48	52	51	52	51	49	53	50	53	55	51	58	1.6	0.05
P, g/kg DM	2.8	3.0	2.9	2.8	3.1	3.2	3.1	3.1	3.0	3.1	3.1	3.1	3.0	3.2	3.1	3.4	0.05	0.01
Zn, ppm	23.5	24.0	21.5	26.0	24.5	24.5	27.5	27.0	24.0	23.5	26.0	26.5	24.5	24.0	28.5	27.0	1.30	0.03
Mn, ppm	17.0	16.5	14.0	14.5	15.0	15.5	15.0	14.5	13.0	12.5	13.0	12.5	15.5	15.0	13.5	15.0	0.52	0.02
Fermentation Products																		
pH	5.80	3.80	3.80	3.85	5.75	3.80	3.80	3.80	5.55	3.70	3.80	3.80	5.85	3.70	3.80	3.75	0.07	<0.0001
Acetic acid, g/kg	1.0	14	12	13	0.8	11	13	12	0.6	11	12	13	0.6	11	12	13	1.0	<0.0001
Lactic:acetic ratio	0.79	4.28	5.46	5.31	0.55	6.41	5.54	5.35	0.55	6.05	5.68	5.47	0.72	6.52	5.88	5.81	0.45	<0.0001
Amm-N, g/kg total N	25	40	55	60	15	40	55	60	10	40	50	60	15	40	50	60	2.8	<0.0001
VFA score	1.57	8.18	8.67	8.78	1.57	8.92	8.84	8.67	1.57	8.73	8.88	8.87	1.57	9.05	8.96	9.11	0.13	<0.0001

[1] Treatment = Fungicide treatments were control plot (CON, with no application of fungicide), V5 (with 1 application at V5), R1 (with 1 application at R1), and V5+R1 (with 2 applications at V5 and R1).

[2] Fixed effects of (TP) effect due number of days ensiled postharvest.

[3] Soluble protein, as percentage of crude protein

[4] Log transformation *P*-values presented, non-transformed data presented.

Figure 4.1. Lignin, g/kg DM, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point interaction P = 0.04.

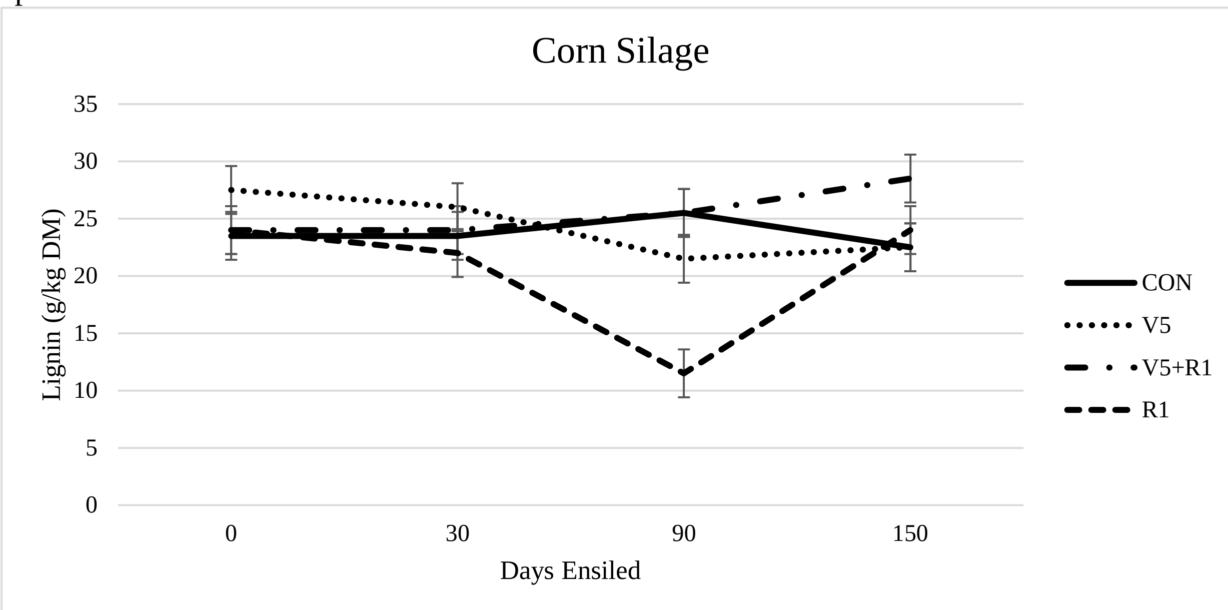


Figure 4.2. Water soluble carbohydrates, g/kg DM, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point interaction P = 0.03.

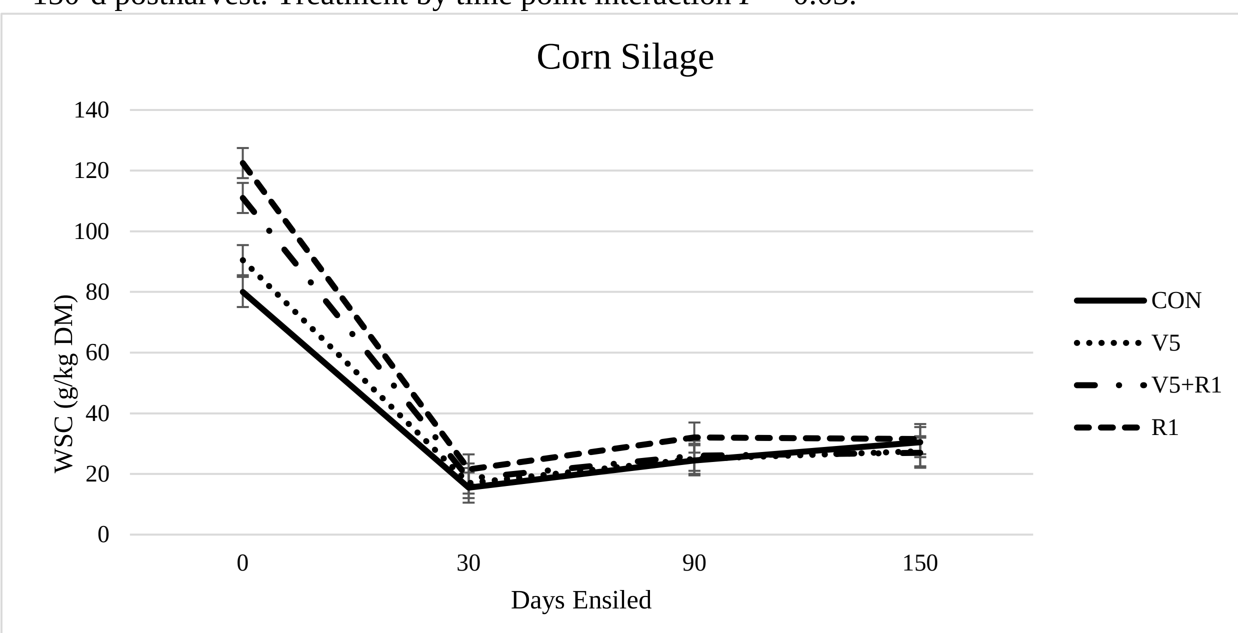


Figure 4.3. Sodium, g/kg DM, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point interaction $P < 0.0001$.

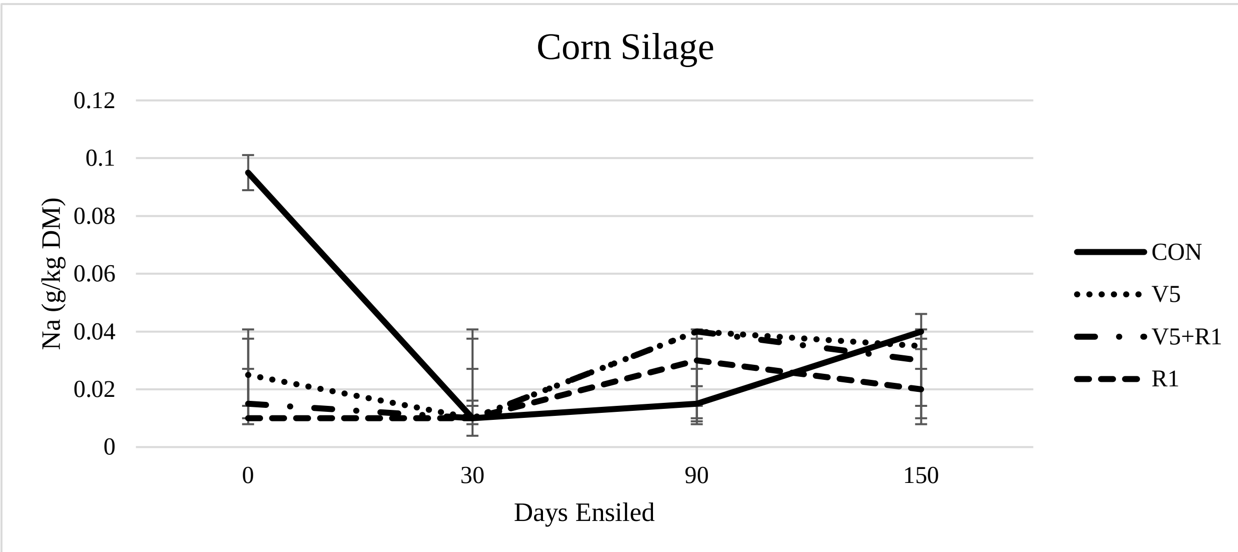


Figure 4.4. Iron, ppm, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point interaction $P = 0.03$.

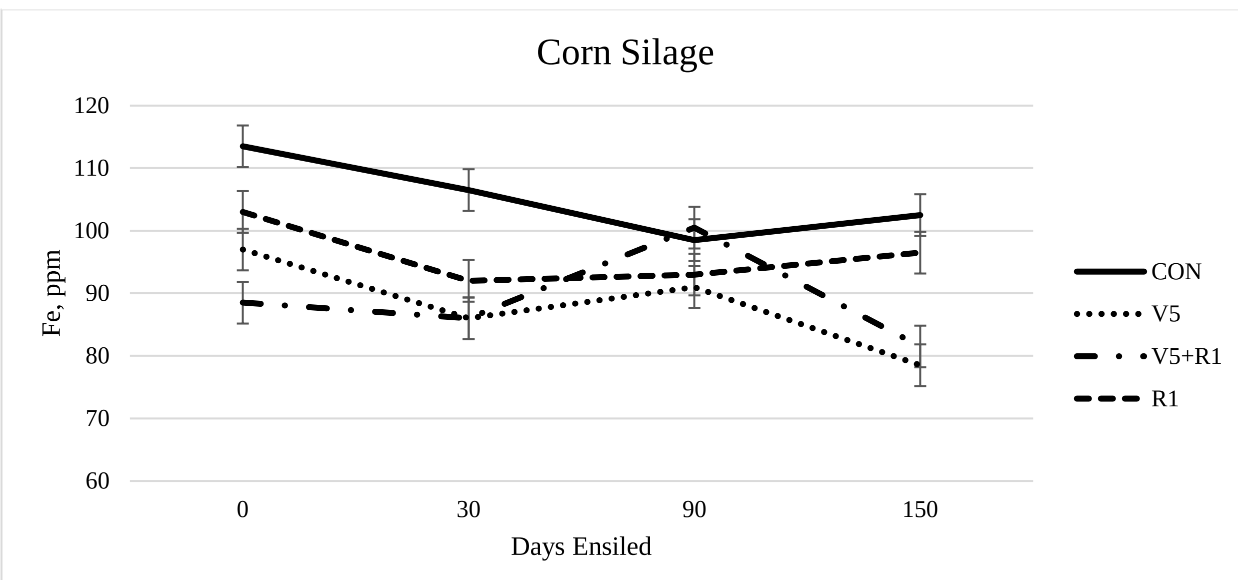


Figure 4.5. Lactic acid, g/kg DM, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point P = 0.03

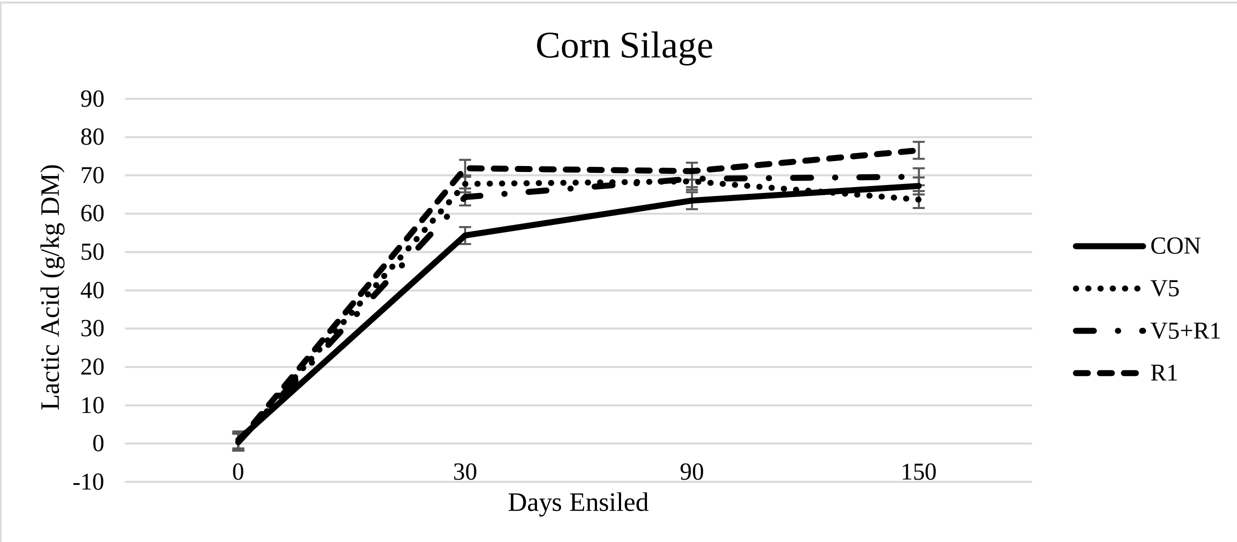


Figure 4.6. Total acid, g/kg DM, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point interaction *P* = 0.005.

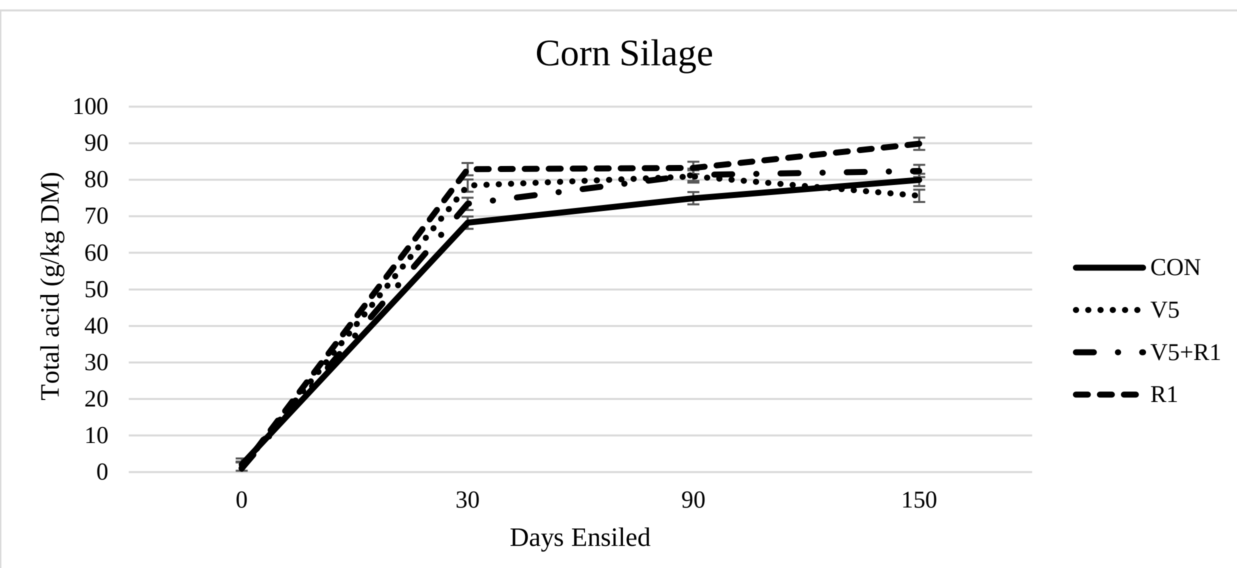


Figure 4.7. Ammonia, g/kg DM, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point interaction *P* = 0.02.

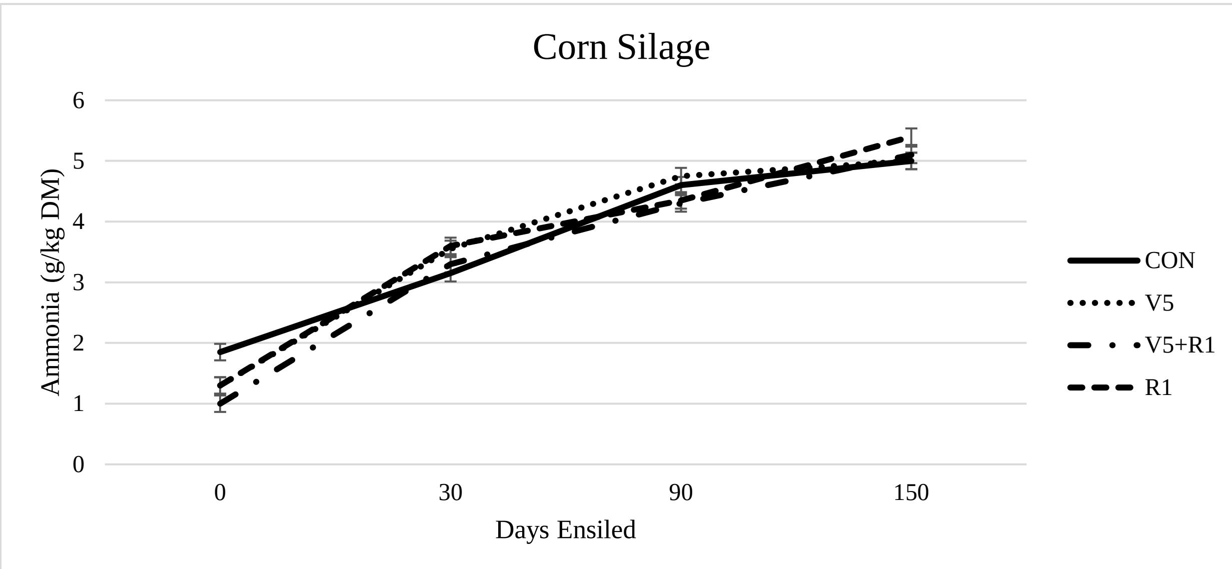


Figure 4.8. Milk kg/MT DM for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0, 30, 90, or 150-d postharvest. Treatment by time point interaction *P* = 0.04.

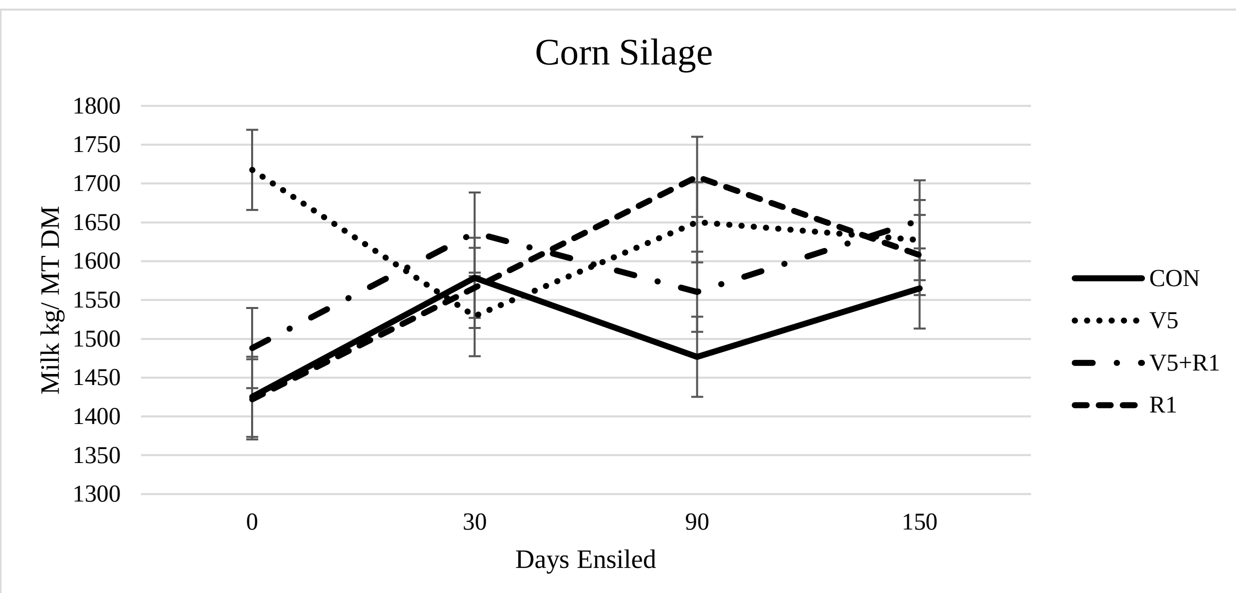
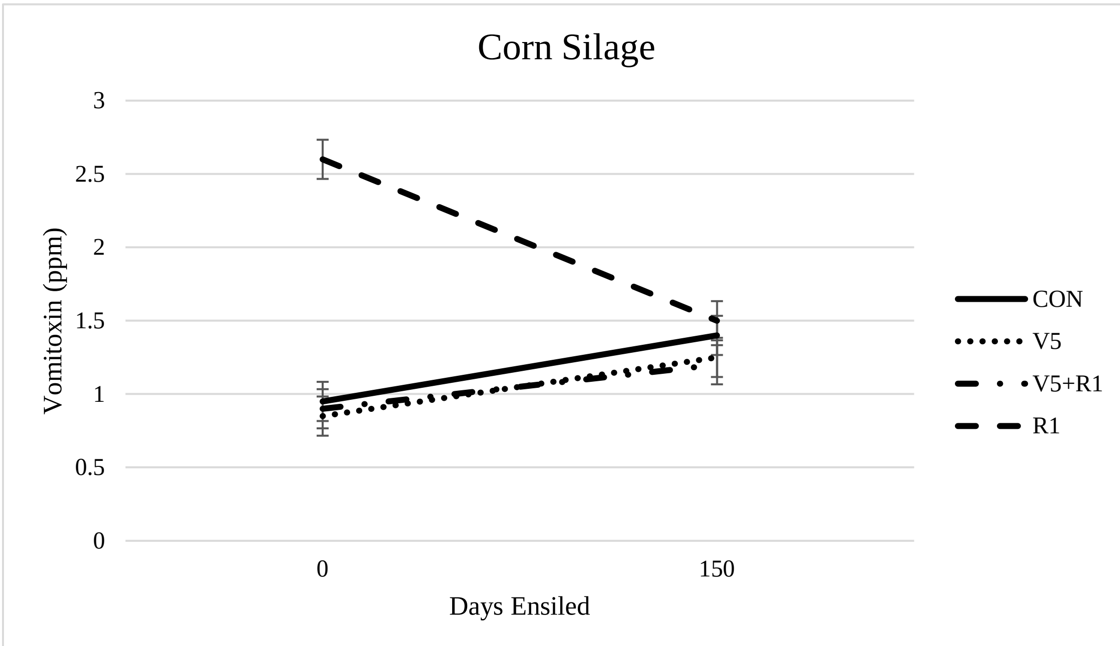


Figure 4.9. Vomitoxin, ppm, for corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at R1 (**R1**), or two applications of foliar fungicide at V5 and R1 (**V5+R1**) ensiled for either 0 or 150-d postharvest. Treatment by time point interaction *P*= 0.01.



CHAPTER V

OVERALL SUMMARY AND CONCLUSIONS

In order to feed the projected number of humans by 2050, crop producers and dairy producers need to find techniques to maximize efficiency and thereby, limit food insecurity. When growing crops, fungal infection on corn is a threat to food security, by reducing yield, increasing the fibrous content of the feedstuff, and releasing mycotoxins within the plant. A better understanding of how fungicide application on corn influences the nutrient profile of the corn plant and limits the negative impact of disease could be a beneficial means in helping to reach the goal of feeding the world.

Thinking about how the world will be fed, diets cannot be formulated using grains alone, protein needs to be included for a balanced diet. The milk and meat from dairy cows is one option where food can provide a valuable source of protein for humans. Improving the feed conversion of dairy cattle by limiting the fibrous content of corn silage and improving the fermentation process of the ensiling when using corn silage with foliar fungicide application in the field could help reduce the demand for corn needed to supply the same volume of milk by dairy producers.

This problem will not be fixed with just one solution, by one person. Instead, it will take teams of investigators and cooperation by people of all backgrounds: crop producers, microbiologists, animal scientists, agronomists, veterinarians, crop scientists, chemists, policy makers, and everyday people making decisions on the products they are buying and consuming.

Indeed, we have made great progress this year in helping to answer some of the questions and provide a better idea of what is happening:

- Applications of fungicide at V5 on corn, ensiled as corn silage fed to dairy cows had greater fat yield than applications of fungicide on corn at V8,
- Applications of fungicide at V5 on corn, ensiled as corn silage fed to dairy cows had greater 3.5% FCM and ECM yield than applications of fungicide at V8,
- Applications of fungicide on corn ensiled as corn silage fed to dairy cows had greater concentration of lactose than cows fed untreated corn silage,
- Created an experimental protocol to systematically remove corn plants from the field and identify nutrition differences between fungicide treated and untreated corn,
- Applications of fungicide on corn had less yellow leaves and taller plants than untreated corn,
- Two applications of fungicide at V5 and R1 reduced NDF and ADF content in corn leaves, but increased the lignin content in the stalks,
- Created an experimental protocol to systemically test the effect of fungicide applications on the corn silage and the fermentation process,
- Applications of fungicide on corn ensiled as corn silage decreased the dry matter compared to untreated,
- Applications of fungicide on corn ensiled as corn silage increased the CP content, WSC concentration, and lactic acid content compared to untreated,
- Applications of fungicide at R1 on corn ensiled as corn silage resulted in decreased lignin concentration,
- Applications of fungicide V5 on corn ensiled as corn silage projected the greatest milk kg/ metric ton DM compared to other applications treatments,

- Developed an idea to remove corn roots from the field in an identical manner,
- Applications of fungicide on corn may alter root growth and nutrient uptake compared to untreated corn.

But, more work still needs to be done. It is still difficult to determine at which time during the growing period of corn it is most beneficial for fungicide application for silage production and feeding of ruminants. In the future, a better understanding of the mechanism, the enzymes affected, and how applications at different times under different environmental conditions would only enhance the conversation, and support global food production. Furthermore, a better understanding of the digestibility by cattle of different parts of the corn plant and how fungicide application alters the digestibility may assist in the fungicide decision process. For example, what part of the corn plant is hindering the digestibility of corn silage for cows, the stalk, the ear, the leaf? And, furthermore how applications of fungicide may alter the digestibility?

During the summer of 2016, we will be investigating the effects of fungicide application at R1 on BMR corn and leafy corn, two different genetic hybrids of corn. This summer we will look to answer if applications of fungicide on corn plants limit the oxidative stress in treated plants compared to untreated plants. Moreover, insight in if different genetic hybrids react differently to fungicide treatment. Corn plant collection and corn silage silos will be replicated in a similar manner to summer of 2015 described in this thesis. It is our hope, the corn from summer of 2016 will be fed to cows as silage in the spring/summer of 2017. Each study will only aid in helping to ensure a stable food production for a growing population in years to come.

APPENDIX A

Using thermal imaging to evaluate corn silage quality

Kalebich, C., M. Carroll, and F. Cardoso

INTRODUCTION

Silage quality is the cornerstone in a nutrient rich, balanced dairy diet. A diet with high quality silage may not need to be supplemented with as many other ingredients in order to provide a balanced diet, as incorporating a low quality silage into the diet may require. Therefore, development of repeatable, on farm tests for silage quality evaluation could help nutritionists and producers better understand the feedstuff they are working with.

Temperature of silage can provide a beneficial indicator for assessing silage quality, especially, throughout the ensiling process. Observations in temperature change provide key insight into the speed of primary fermentation, the occurrence secondary fermentation by yeasts and molds, and the level of aerobic stability of corn silage (Cherney and Cherney, 2003). For the production of stable silage, it is recommended the temperature of corn silage at ensiling to be -9°C and -7°C above ambient temperature (Mahanna and Chase, 2003). Increases in silage temperature at ensiling may arise due to an assortment of variables including: slow filling of the silo or bag, poor compaction, low moisture of the crop, or ensiling an overly mature crop (Mahanna and Chase, 2003). Due to the exposure of air when feeding, aerobic instability is demonstrated by measureable increases in heat production of the silage (Pahlow et al., 2003). Rise in silage temperature can result from oxidative reactions occurring because of extended respiration or growth of yeast, mold or bacteria (Mahanna and Chase, 2003), which metabolize sugars, and lactic acid, acetic acid, and ethanol within the corn silage as substrates (Pahlow et al., 2003). Borreani and Tabacco (2010) collected corn silage samples from three areas on the face of silo (the core, the peripheral, and molded areas) and collected environmental temperature and the temperature behind the face of silage from 54 dairy farms in Italy. Compared to an individualized corn silage reference temperature developed by the authors, silage temperature was 9.9°C and 13.3°C on average greater for corn silage from the peripheral and mold areas when compared with corn silage from the core (Borreani and Tabacco, 2010). Furthermore, an aerobic deterioration value greater than 5°C above the reference index was associated with a yeast count higher than 5 log cfu/g in corn silage from both the peripheral areas and molded samples (Borreani and Tabacco, 2010). Being able to locate on the face of a silo where secondary fermentation occurred, and therefore, feed around it, would allow producers to more consistently include higher quality silages in the diet.

The use of infrared technology to assess corn silage quality allows producers the ability to see where heat is being produced within the silage. Few studies have been published evaluating the use of this technology in helping determine silage quality. Felton and DeVries (2010) observed the temperature of a TMR pile in a cow's feed bunk using a thermal camera, and a forage temperature probe. The authors found the temperature reading on the thermal camera positively correlated with the reading on the temperature probe. Additionally, a survey of 109 dairy farms in Brazil evaluated the temperature of corn silage using infrared technology, and found ambient temperature to be correlated with the infrared measurements of corn silage (Schmidt et al., 2015). The authors concluded the use of infrared technology is useful to predict heat spots, but must be carefully interrupted, as the environmental temperature can influence the reading (Schmidt et al., 2015).

The growth of fungus in the field on corn crops can result in decreased dry matter as the fungal infection attacks the nutrients of the plant cells locally killing tissue. Application of foliar fungicide on corn ensiled as corn silage may increase fermentation and the aerobic stability when exposed to air. Haerr et al. (2015) reported three applications of fungicide on corn silage resulted in the most stable corn silage after 38 h exposed to air when compared to corn silage from corn with no application of fungicide, one application of fungicide, or two applications of fungicide.

The objective of our experiment was to evaluate the quality of corn silage treated with foliar fungicide using an infrared camera on the face of the silo, the side of the silo, and TMR in the feed bunk. We hypothesized the corn silage with three applications of foliar fungicide on corn ensiled as corn silage would result in the lowest average temperature.

MATERIALS AND METHODS

The corn hybrid planted was the Pioneer 1498 CHR RR + Pioneer 1498 RR refuge 2014 Variety (Johnston, IA), the purpose of which is silage. Treatments of foliar fungicide on corn silage were as follows: corn receiving no foliar fungicide application **(CON)**; corn received one application of pyraclostrobin ($C_{19}H_{18}ClN_3O_4$) and fluxapyroxad ($C_{18}H_{12}F_5N_3O$) (**PYR+FLUX**), foliar fungicide (Priaxor; BASF Corp.) at corn vegetative stage 5 where emergence of the fifth leaf is visible (**V5**; Mueller and Pope, 2009); corn received two applications of foliar fungicides, PYR+FLUX at corn vegetative stage 5, and PYR+FLUX at corn vegetative stage 8 when the eighth leaf is visible (**V5/V8**; Mueller and Pope, 2009); corn received three applications of foliar fungicides, PYR at corn vegetative stage 5, PYR at corn vegetative stage 8, and a mixture of pyraclostrobin ($C_{19}H_{18}ClN_3O_4$) + metconazole ($C_{17}H_{22}ClN_3O$) foliar fungicide (**MET**; Headline AMP®; BASF Corp.) at corn reproductive stage 1 or when the silks are fully extended (**V5/V8/R1**; Mueller and Pope, 2009).

Upon reaching the 3/4 milk stage of corn development, harvest for CON and V5 occurred on September 2, 2014 and on September 3, 2014 for V5/V8 and V5/V8/R1. The DM for CON, V5, V5/V8, and V5/V8/R1 measured 31.0, 33.3, 30.2, and 31.7%, respectively. Chopped corn was transported by H&P forage wagons from the field to scale where each full wagon was weighed and recorded. Once at the storage site, chopped corn material was ensiled in 2.74-m diameter horizontal bags using an AG bagger. The calculated dry matter of the silage from each treatment allowed for individual adjustments to the bagger, preserving each bag in a uniform manner. Additionally, an inoculant (Silo King, Agri-King) was added at a rate of 115 g for 1000 kg of corn to better preserve the corn silage. Horizontal silos were filled with one treatment, then a non-treatment corn silage put in between treatments, with a second treatment filling out the silo. Bags were aligned in the north and south direction; corn silage in control and V5/V8 faced south and corn silage in V5 and V5/V8/R1 faced north. Corn silage was ensiled for at least 245 days before opening.

Thermal images were taken for each treatment biweekly at 1500 h using an infrared camera (Fluke Thermal Imager, IR Flexcam, Everrett, Washington). Thermal images of the face of the silo and the TMR in the bunk were collected for 11 individual days and of the side of the silo for 10 individual days. Images of TMR were taken from 1 m above the bunk, 1 hr after feed delivery. The camera is able to capture the average temperature, the minimum temperature, and the maximum temperature of the image located in the frame. Red, yellow, and blue are used to indicate the amount of heat being given off. The more red the color, the warmer the temperature reading and more heat production. Blue represents the coolest temperature readings within the frame. To minimize variation, the same evaluator was used to take images each day. Data for mean environmental temperature for Champaign-Urbana, IL was collected from the state climatologist office for Illinois (Illinois State Water Survey, Prairie Research Institute, Champaign, IL). Averages and standard deviation of corn silage temperature are presented in the subsequent tables and figures.

RESULTS

Overall, the average temperature of the face of the silo was not different for applications of foliar fungicide on corn ensiled as corn silage (Table A.1). Furthermore, the average temperature for the face of the silo for corn silage in each treatment seemed to follow the variation in the environmental temperature (Figure A.1). The average temperature of the side of the bag for treatment silage was not different for corn silage with foliar fungicide application compared to corn silage with no application (Table A.2). The average temperature for the side of corn silage seems to followed environmental temperature by June 30, prior to this date the temperature of treatment corn silage seemed to vary more (Figure A.3). The corn silage that is closest to the ground was cooler in temperature when compared to corn silage along the top of the silo (Figure A.4). When mixed and fed to cows, the average temperature of TMR in the bunk was not different for cows fed diets containing corn silage with foliar fungicide application compared to cows fed diets containing corn silage with no application (Table A.3). No differences were observed in the average temperature of TMR in the bunk at each day of collection (Figure A.5). Using thermal imaging, total mix ration in V5/V8/R1 may have been a bit warmer when compared with other TMR in CON, V5, and V5/V8 (Figure A.6). Of the thermal images taken and collected, the maximum average temperature of corn silage in control was the hottest 5 of 11 camera frames, and corn silage in V5 was the hottest for 5 of the 11 camera frames, together totaling 10 of the 11 camera frames. Corn silage in V5/V8/R1 was the hottest once, and corn silage in V5 was never the hottest. (Figure A.7).

DISCUSSION

The objective of this study was to observe changes in the temperature of corn silage treated with foliar fungicide application compared with corn silage with no application of foliar fungicide. Corn silage in CON and in V5/V8 had slightly greater mean face temperatures when compared with corn silage in V5 and in V5/V8/R1 (Figure A.1). Furthermore, corn silage in CON and V5/V8 had the highest average temperature more frequently when compared with corn silage in V5 and V5/V8/R1 (Figure A.7). Additional heat production of corn silage in CON and V5/V8 may have been due to increased concentration of molds and yeasts on the plant material when ensiled compared with corn silage in V5 and V5/V8/R1. Schmidt et al. (2015) concluded using temperature of corn silage as predictor of mycotoxin concentration was not accurate. In the future, additional testing for molds and yeast on treatment corn silage should be conducted to make more conclusive statements. Furthermore, slightly higher temperature of corn silage in CON and in V5/V8 when compared with corn silage in V5 and in V5/V8/R1 may be the result of bag placement in regards to the sun's rays. Feed temperatures in the bunk (Felton and DeVries, 2010) and the infrared camera temperature readings of silos (Schmidt et al., 2015) have been correlated with ambient temperature. On the west side of the treatment silage bags was a covered hay storage building, which may have blocked the direct afternoon rays of the sun on the face of corn silage in V5 and in V5/V8/R1. Therefore, direct sun exposure on corn silage in CON and V5/V8 at the time of imaging may have overestimated increases in temperature. For this reason, Schmidt et al. (2015) cautioned dairy producers to carefully interpret infrared temperature readings.

Thermal images on the face of the silo (Figure A.2) and the side of the silo (Figure A.4) reveal where corn silage is producing heat. Corn silage along the top and the side in the face frame tend to be more saturated with red color than corn silage in the core of the same images. Borreani and Tabacco (2010) found temperature of corn silage in the peripheral area of the silo to be 9.9°C warmer than corn silage in the core, with more than 55% of the peripheral samples recording a temperature greater than 30°C. The same study suggested the exponential growth of microbes along the peripheral could contaminate corn silage during feed out (Borreani and Tabacco, 2010). Looking at the side images of corn silage, corn silage along the bottom of the silo was more yellow in color. Our hypothesis is the cool ground insulated the temperature of corn silage along the bottom of the silo. Furthermore, it may have been a greater concentration of water in corn silage along the bottom of the silo may have reduced the temperature relative to corn silage in the upper part of the silo. Moisture concentration within a silo is directly or indirectly related to gravity, silage density, pressure, fermentation, and plant respiration (Muck et al., 2003).

No differences in temperature of TMR was observed for cows fed diets containing corn silage treated with foliar fungicide compared to diets including control corn silage. Felton and DeVries (2010) reported adding water to the TMR at feed delivery reduced the initial temperature, but post-delivery, the temperature of TMR with the greater water concentration increased relative to TMR with lower concentrations of water. Warmer spots of TMR in V5/V8/R1 (Figure A.6), as indicated by red color in the thermal image, could have been the result of small variation in the water concentration of the feedstuff.

Overall, foliar fungicide treatment on corn ensiled as corn silage did not seem to effect the temperature of corn silage or TMR. Including more variable in the future would allow for a more definitive statement on using the thermal camera to analyze silage quality.

Future Ideas

In the future, an observational study using thermal imaging and corn silage should be replicated. The experiment should involve corn silage in horizontal bags, as done in this study. At ensiling, the temperature of corn silage would be recorded and samples for corn silage from treatment. After opening bags for feeding, temperature readings would be taken using the thermal camera before and after feeding of the silo, as well as, a temperature probe inserted into the silo's face. Temperature for obviously molded spots would also be recorded using both the camera and the temperature probe. Collection of corn silage before feeding and after feeding, and corn silage from obviously molded places, and sent for nutrient analysis. The purpose of the study would be to see if fungicide application on corn silage limited the development of molds and yeasts within the silo compared with corn silage without application. The use of a drone equipped with a thermal camera would allow us to gather thermal images of corn silage from a bird's eye view, to see if foliar fungicide affects temperature gradients during the feed out period.

CONCLUSION

Overall, foliar fungicide treatment on corn ensiled as corn silage did not seem to affect the temperature of corn silage or TMR. Including more variable in the future would allow for a more definitive statement on using the thermal camera to analyze silage quality.

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TABLES AND FIGURES

Figure A.1. Average temperature of the silo face of the corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at V5 and one application at V8 (**V5/V8**), or one application of foliar fungicide at V5 and one application at V8 (**V5/V8/R1**).

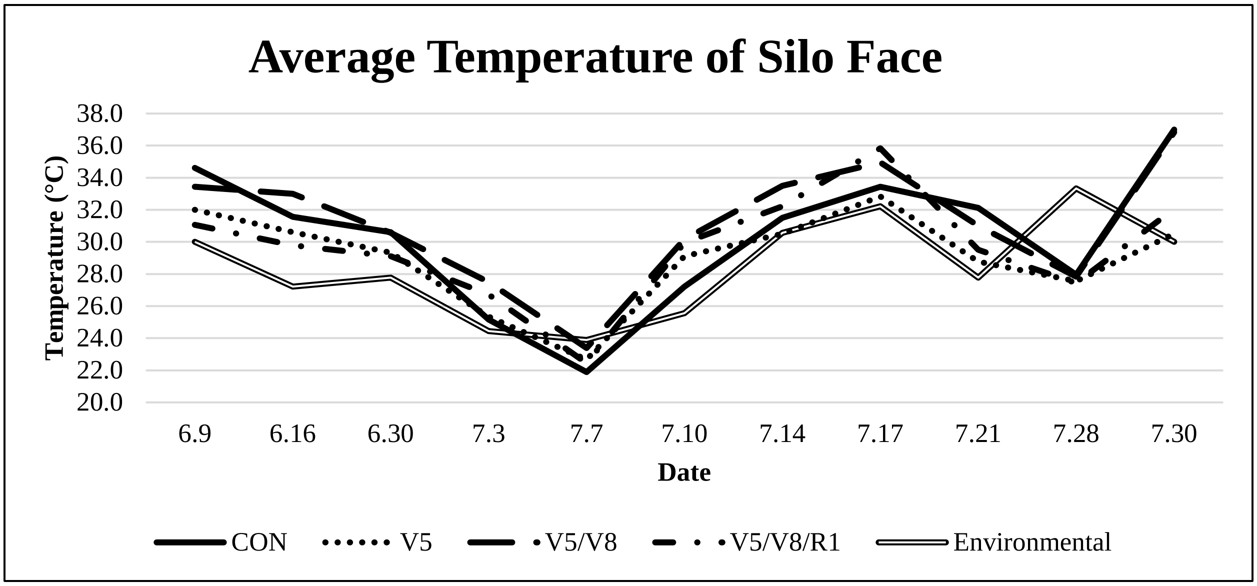


Figure A.2. Representative images of the silo face of the corn silage in (**CON; A**), one application of foliar fungicide at V5 (**V5; B**), one application of foliar fungicide at V5 and one application at V8 (**V5/V8; C**), or one application of foliar fungicide at V5 and one application at V8 (**V5/V8/R1; D**).

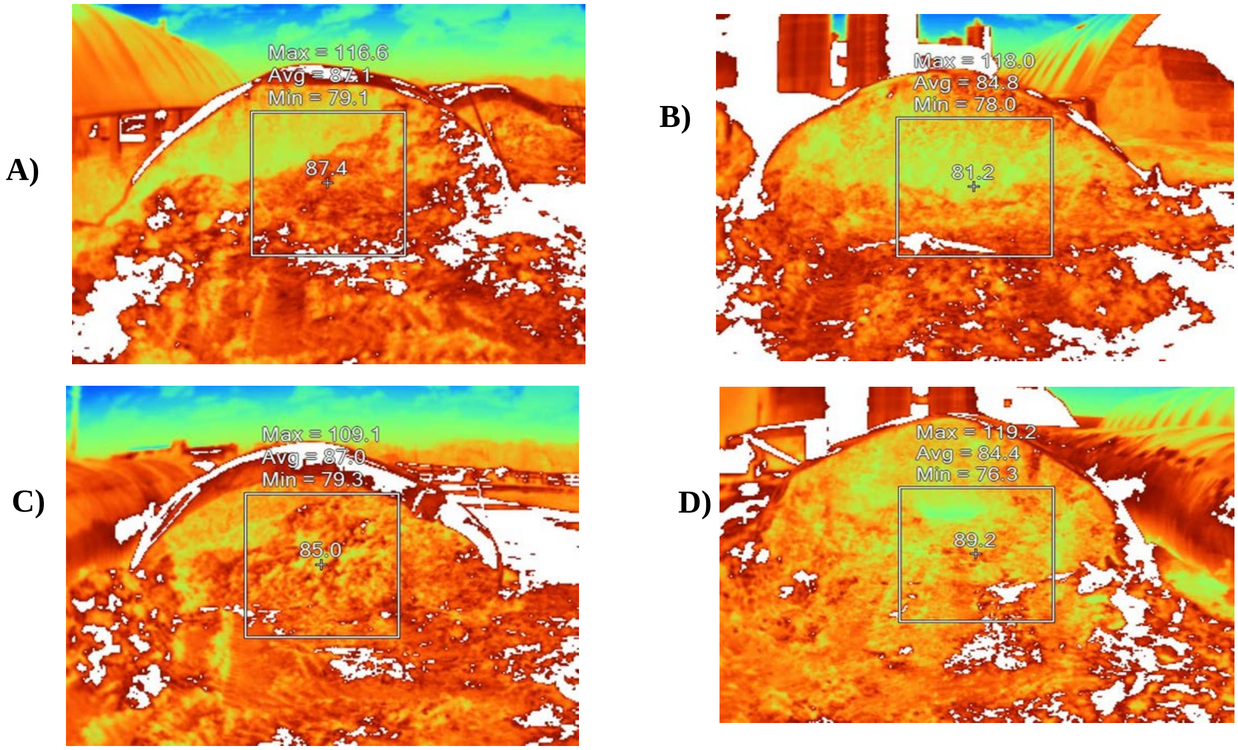


Table A.1. Average temperature and standard deviation of front of silo of corn silage treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**), or three applications of foliar fungicide (**V5/V8/R1**), and environmental temperature.

	Treatment[1]				
	CON	**V5**	**V5/V8**	**V5/V8/R1**	**Environmental**
Temp. (°C)	30.3	29.0	31.1	29.6	28.4
SD	4.4	2.9	3.8	3.4	3.1

[1] Treatment = Dietary treatments were CON (with no application of fungicide), V5, (with one application of fungicide at V5), V5/V8 (with two applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1). Average and standard deviation of eleven images per treatment.

Figure A.3. Average temperature of the side of silo of the corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at V5 and one application at V8 (**V5/V8**), or one application of foliar fungicide at V5 and one application at V8 (**V5/V8/R1**).

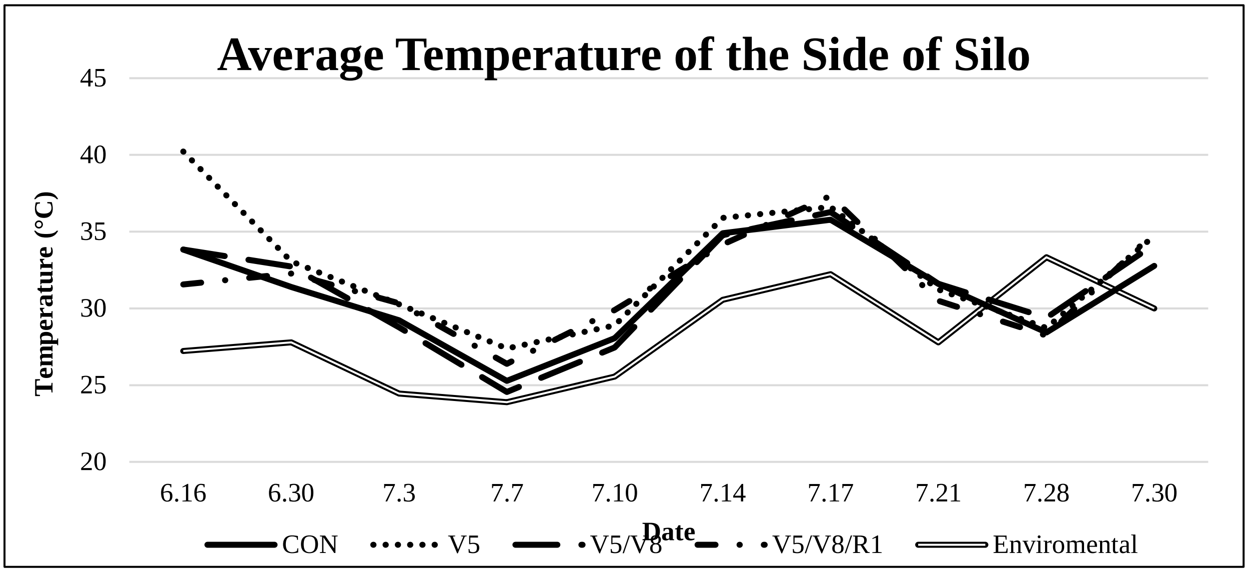


Figure A.4. Representative images of the side of the silo of the corn silage in (**CON; A**), one application of foliar fungicide at V5 (**V5; B**), one application of foliar fungicide at V5 and one application at V8 (**V5/V8; C**), or one application of foliar fungicide at V5 and one application at V8 (**V5/V8/R1; D**).

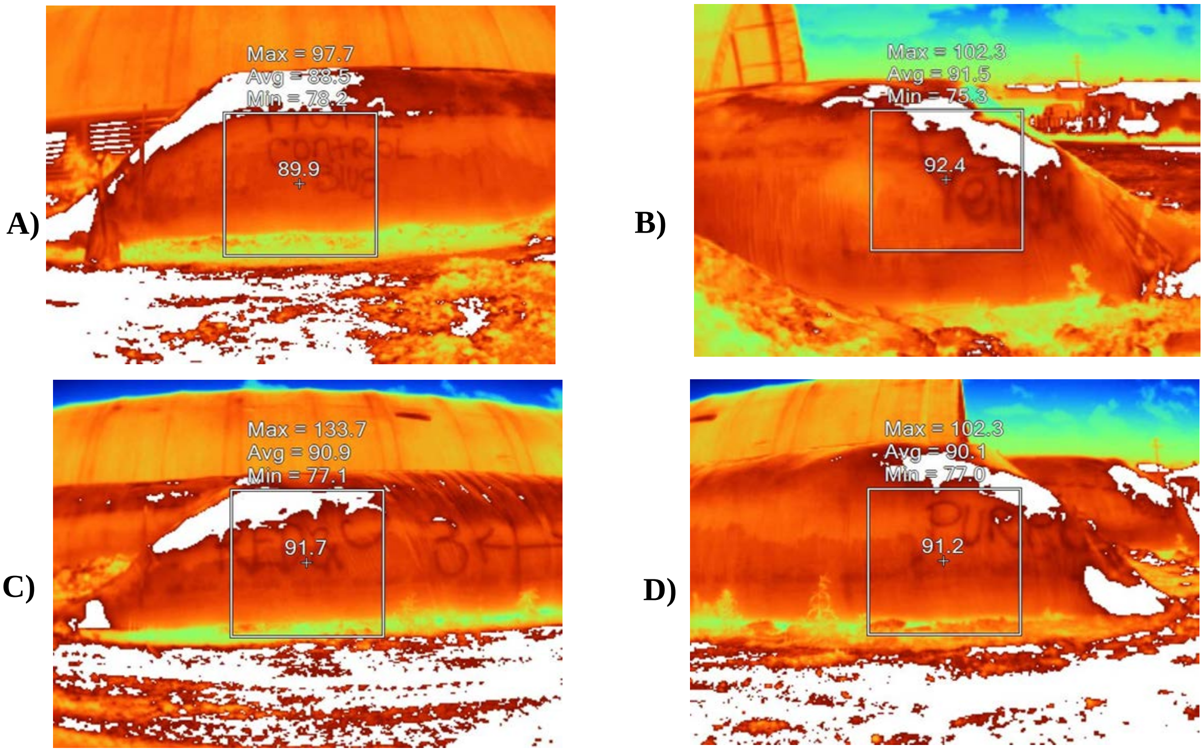


Table A.2. Average temperature and standard deviation of the side of silo of corn silage treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**), or three applications of foliar fungicide (**V5/V8/R1**), and environmental temperature.

	Treatment[1]				
	CON	**V5**	**V5/V8**	**V5/V8/R1**	**Environmental**
Temp. (°C)	31.1	32.7	31.4	31.6	28.4
SD	3.3	4.1	3.7	3.3	3.1

1 Treatment = Dietary treatments were CON (with no application of fungicide), V5, (with one application of fungicide at V5), V5/V8 (with two applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1). Average and standard deviation of ten images per treatment.

Figure A.5. Average temperature of the TMR in the bunk for cows fed corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at V5 and one application at V8 (**V5/V8**), or one application of foliar fungicide at V5 and one application at V8 (**V5/V8/R1**).

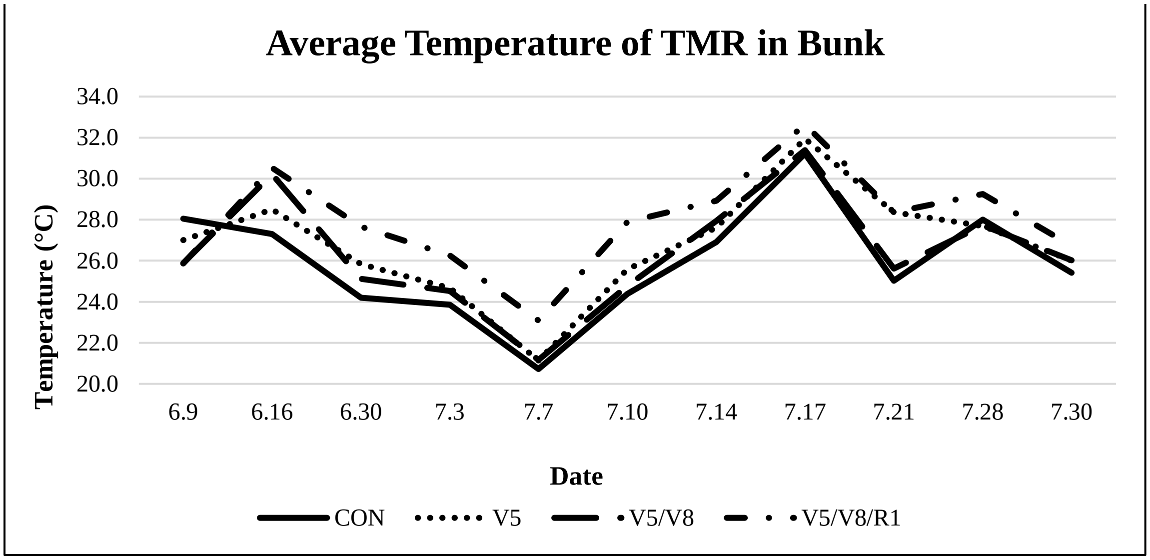
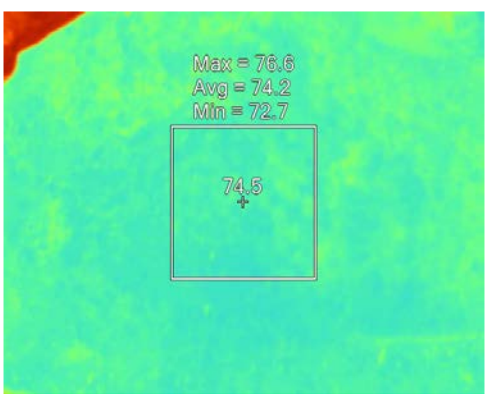
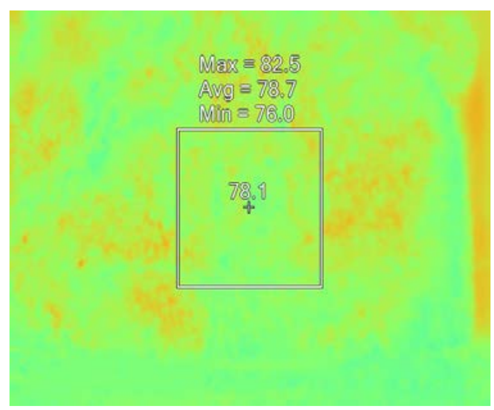


Figure A.6. Representative images of the TMR in the bunk for cows fed corn silage in (**CON; A**), one application of foliar fungicide at V5 (**V5; B**), one application of foliar fungicide at V5 and one application at V8 (**V5/V8; C**), or one application of foliar fungicide at V5 and one application at V8 (**V5/V8/R1; D**).

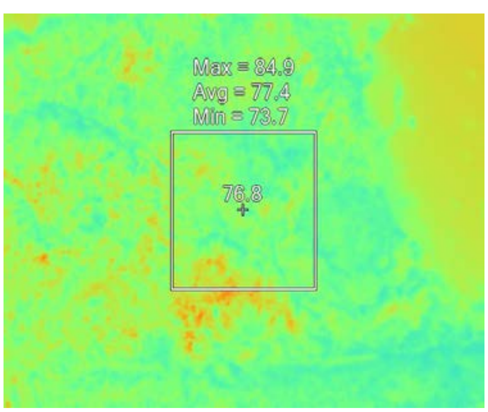
A)



B)



C)



D)

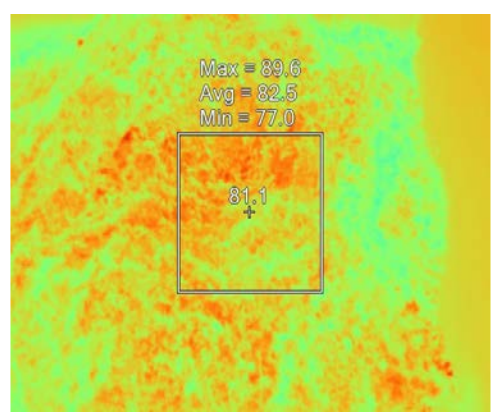
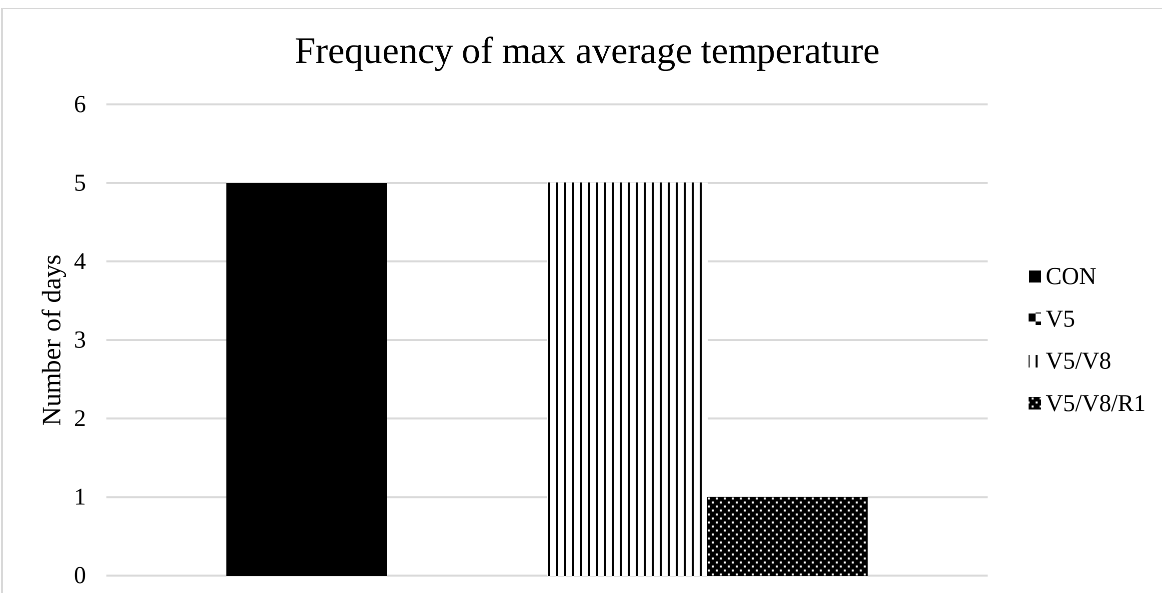


Table A.3. Average temperature and standard deviation of TMR in bunk for cows fed corn silage treated with no applications of foliar fungicide (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**), or three applications of foliar fungicide (**V5/V8/R1**)

	Treatment[1]			
	CON	**V5**	**V5/V8**	**V5/V8/R1**
Temp. (°C)	25.9	26.8	26.4	27.9
SD	2.8	2.7	2.8	2.5

1 Treatment = Dietary treatments were CON (with no application of fungicide), V5, (with one application of fungicide at V5), V5/V8 (with two applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1). Average and standard deviation of ten images per treatment.

Figure A.7. Frequency of days corn silage in (**CON**), one application of foliar fungicide at V5 (**V5**), one application of foliar fungicide at V5 and one application at V8 (**V5/V8)**, or one application of foliar fungicide at V5 and one application at V8 (**V5/V8/R1**) was the highest average temperature.



Appendix B

Holstein *In-situ* dry matter digestibility of foliar fungicide treated corn silage

Kalebich, C., and F. Cardoso

INTRODUCTION

Estimation of digestibility of a feedstuff in the rumen using an *in situ* technique is a valuable tool for estimating the nutritional value of feedstuffs (Van Milgen et al., 1991). Polyester bags are filled with dried forage, either ground or unground, to measure the disappearance of the feedstuff for various time intervals. A main advantage to using the *in situ* technique as compared to the *in virto* technique for estimating the degradability of a feedstuff, is the digestive process of the bags is conducted within a live animal (Cherney and Cherney, 2003). Yet, lack of precision and standardization among researchers using the *in situ* technique adds difficulty in discussing results in a meaningful way (Vanzant et al., 1998). Discrepancy for *in situ* measurements may occur because of variation in the animals, substrate characteristics, bag size and porosity, incubation and removal procedures, and modeling mistakes (Vanzant et al., 1998; Cherney and Cherney, 2003).

Whole plant corn silage is a high energy forage fed to dairy cattle. In recent years, dairy producers and nutritionists have placed a greater emphasis on the nutritive quality of the feedstuff and a better understanding of factors affecting. Many have evaluated the *in situ* digestibility of corn silage for a better understanding of the degradability within the rumen and the incorporation in the diet. Researchers have evaluated the effect of time of harvest (Philippeau and Michalet-Doreau, 1998; Bal et al., 2000), corn silage hybrids (Philippeau and Michalet-Doreau, 1998; Bal et al., 2000) mechanical processing of plant material (Bal et al., 2000), NDF concentration (Bal et al., 2000), and storage of corn silage (Peyrat et al., 2014) on the dry mater degradability using the *in situ* technique.

The growth of fungus in the field may increase the fibrous content within the plant cells and decrease the dry matter degradability within the rumen, therefore, negatively affecting the nutritive quality of the feedstuff. A consequence of fungus infection, corn silage from infected corn plants resulted in increased concentration of ADF and NDF and decreased *in vitro* NDF digestibility when compared to uninfected corn silage (Quieroz et al., 2012). Furthermore, inoculation of Northern Leaf Blight on corn plants ensiled as corn silage decreased the true dry matter digestibility when fed to sheep compared to sheep fed control corn silage (Wang et al., 2010).

Foliar fungicide applications on corn assist in managing fungal outbreaks on the corn plant when the crop is suffering from disease pressure (Wise and Mueller, 2011). Haerr (2015) evaluated the effects of increasing foliar fungicide applications on corn ensiled as corn silage on the ruminal *in situ* digestibility of corn silage, using Holstein cannulated cows. Applications of foliar fungicide on corn, ensiled as corn silage increased the digested DM portion of corn silage when compared to control (Haerr, 2015). Therefore, applications of foliar fungicide on corn ensiled as corn silage may decrease the negative effect of fungal disease on the plant and increase the nutritive quality of the feedstuff. The objective of this experiment was to evaluate the effect of foliar fungicide applied at various times during the growth and development on ruminal *in situ* DM degradability.

MATERIALS AND METHODS

Plant Material

The corn hybrid planted was the Pioneer 1498 CHR RR + Pioneer 1498 RR refuge 2014 Variety (Johnston, IA), the purpose of which is silage. This variety of corn is marketed for drought tolerance, high yields and high digestibility among ruminants. The hybrid genetics allow for resistance towards Gray Leaf Spot (caused by the disease *Cercospora zeae-maydis*), Northern Leaf Blight (caused by the fungus *Exserohilum turcicum)*, Fusarium Ear Rot (caused by the fungus *Fusarium verticillioides)*, and corn earworm (*Helicoverpa zea*). Corn seeds were planted at a latitude and longitude of 40°04'58.8"N 88°13'08.4"W, on May 19, 2014 and treatments were randomly assigned to one of four 0.8-ha plots. Treatments were as follows: corn receiving no foliar fungicide application **(CON)**; corn received one application of pyraclostrobin and fluxapyroxad (**PYR+FLUX**), foliar fungicide (Priaxor; BASF Corp.) at corn vegetative stage 5, where emergence of the fifth leaf is visible (**V5**; Mueller and Pope, 2009); corn received two applications of foliar fungicides, PYR+FLUX at corn vegetative stage 5, and PYR+FLUX at corn vegetative stage 8, where the emergence of eighth leaf is visible (**V5/V8**; Mueller and Pope, 2009); corn received three applications of foliar fungicides, PYR at corn vegetative stage 5, PYR at corn vegetative stage 8, and a mixture of pyraclostrobin + metconazole ($C_{17}H_{22}ClN_3O$) foliar fungicide (**MET**; Headline AMP®; BASF Corp.) at corn reproductive stage 1, when the silks are fully extended (**V5/V8/R1**; Mueller and Pope, 2009).

The fungicide application dates were June 26, July 11, and July 23, 2014. During the growth of the corn plants, foliar disease evaluation of corn plants occurred four separate times. Evaluations occurred at vegetative stage 7 (V7; July 5, 2014), reproductive phase 1 (R1; July 21, 2014), reproductive phase 3 (R3; August 8, 2014) and reproductive phase 4 (R4; August 15, 2014). Ten plants within each treatment were randomly selected for disease evaluation at each time point. Disease severity, as a percentage of leaf area, was estimated using 3 leaves: the ear leaf, 1 leaf above the ear leaf, and 1 leaf below the ear leaf from each selected plant; a method validated by Reis et al. (2007). The same evaluator looked at the plants at each evaluation of plants to minimize possible error.

Upon corn reaching ~32% DM, harvest for CON and V5 occurred on September 2, 2014 and for V5/V8 and V5/V8/R1 on September 3, 2014. Corn was chopped and processed using a New Holland FP240 forage chopper (CNH Industrial, London, United Kingdom). The processor was set to a 1.9 cm theoretical length of chop and a kernel processor was used to improve digestibility of the silage. To estimate the DM of corn silage, a minimum of three samples of chopped corn material from each treatment was composited to estimate the dry matter. The DM for CON, V5, V5/V8, and V5/V8/R1 measured 31.1, 33.3, 30.2, and 31.7%, respectively. H&P forage wagons (H & S Manufacturing Company Inc., Marshfield, WI) transported chopped corn material from the field to scale (Mettler Toledo, Columbus, OH). Once at the storage site, chopped corn material was ensiled in 2.74-m diameter bags using an AG bagger (Ag Bag Systems, St. Nazianz, WI). An inoculant (Silo King, Agri-King, Fulton, IL) was added at a rate of 115 g for 1000 kg of corn to better preserve the corn silage. Corn silage was ensiled for at least 245 d before opening.

Animals

All experimental procedures involving animals was approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee. Three rumen cannulated Holstein cows with 3.6 ± 1.1 parity, 397 ± 52 DIM, and 758.5 ± 46.2 kg of BW were used to estimate the rate of digestibility and degradability of treated corn silage. Cows had feed and water available at all times except at milking. Furthermore, cows were fed once daily at 1500 h and housed in tie stalls, meeting or exceeding space requirements specified in the AG Guide

(FASS, 2010). Cows were fed a mid-lactation diet supplying 100% of the NRC (2001) requirements for energy and all nutrients. The diet included 43% concentrate, and 57% forage, of which 44.95% of the dietary DM consisted of non-treated corn silage.

Sample Collection

The rate and fractions of dry matter digestibility were measured for each of the four corn silages the three rumen-cannulated cows. Nine kilograms of corn silage per treatment were collected from 5 places on the face of each silo bag immediately after feeding. Corn silage was dried in a force air oven for 24-h at 110°C. Only 10 × 20 cm polyester dacron (50-μm bags; Ankom Technology, NY) *in situ* forage bags were used in this study. All bags were labeled according to cow, silage, and replicate, and then placed in the oven for 24-h at 110°C to obtain the dry weight (n = 288). Once dried, bags were filled with intact corn silages. Using a large tub to uniformly mix the corn silage, a scoop was used to gather samples to be divided evenly among bags to decrease sample error. All samples were filled to achieve 20 mg DM/cm^2. Therefore, all bags were filled with approximately 8-g of DM. All bags were sealed twice with a heat sealer. All bags were replicated in each cannulated cow, 3 times at each time point. Before bags were placed into two mesh laundry bags for placement into the ventral rumen of the cows, all bags were soaked in warm water. Three large washers were also placed in the mesh laundry bags to help weigh down the bag in the fluid contents. Bags were pulled at 0, 2, 4, 8, 12, 48, 72, and 96 h. Immediately removed from the rumen, bags were put in a tub of ice water after to stop fermentation. Within a half hour of the time point collection, bags were frozen at -20°C until collection of all bags occurred. After the final collection, bags were frozen for at least 24 h. For final DM analysis, bags were thawed and placed in a washer machine on a gentle rinse for two cycles to reduce microbial content on corn silage. Bags were placed in force air oven 55°C for 72

h. Once completely dry, all bags were weighed again to calculate the amount digested. The number of torn or damaged bags results in missing data replicates (n = 18).

Modeling

Before the data from the digestibility portion was analyzed using PROC MIXED in SAS, the non-linear model of *in situ* digestibility was fit using NLREG. The nonlinear model is based on the portioning of feed such that the fraction of soluble feed (A), fraction of degradable feed (B), and fraction of undegradable feed (C) sum to 1. Using the portion remaining at each time point, estimation of parameters for four parameters occurred for: soluble fraction, digestibility fraction, indigestible fraction, and the rate of digestibility (percentage/hour). With these estimates, the following equation was fit in NLREG:

$$Y = B + C\left(e^{-Vk_d(t-t_1)}\right)$$

where Y = the proportion of corn silage remaining at a specified time point, B = the portion of potentially digestible feed, C = the rumen undegradable feed, t = time point (0, 2, 4, 8, 12, 48, 72, 96 h), k_d = the fractional digestion rate constant, and V = 1 when there is a lag in digestion or V = 0 when there is not a lag in digestion (Ørskov et al., 1980; McDonald, 1981; Van Milgen et al., 1991). With the final estimates, the soluble portion of each data set was calculated using 100% - (B+C). To meet convergence criteria in NLREG, lag for all bags was estimated as zero only after it was determined not helpful in explaining the data. Therefore, eliminated from the model, the modified equation became:

$$Y = B + C\left(1 - e^{-k_d(t)}\right)$$

Statistics

Using the final estimates from NLREG for the four parameters: soluble, digestible, undegradable, and rate of digestibility for corn silage in each treatment, PROC MIXED was used to calculate the probability of associations between treatment and parameters. Treatment and replicate were treated as fixed effects and cow was treated as the random effect. Three contrasts were used. Contrast 1: CON vs. TRT compared control to the average of the treated corn silages (V5, V5/V8, and V5/V8/R1). Contrast 2: V5 vs. V5/V8 compared corn silage sprayed at V5 to corn silage sprayed at V5 and V8. Contrast 3: V5/V8 vs. V5/V8/R1 compared corn silage sprayed at V5 and V8 to corn silage sprayed at V5, V8, and R1. The degree of freedom method was Kenward-Rogers (Littell et al., 1998). The distribution of residuals was evaluated for normality and homoscedasticity. Statistical significance was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

RESULTS

At the first two evaluations, signs of foliar disease from in field evaluations were not present, either due to no disease present or lower than detectable levels of disease for the evaluator. On the third evaluation of foliar disease at R3, corn plants in CON had an average of 2.5 % of leaf area infected with Gray Leaf Spot, and 1% of leaf area infected with common rust; for corn plants in V5 an average of 1% of leaf area was infected with Gray Leaf Spot; for corn plants in V5/V8 an average of 1% of leaf area was infected with common rust, and for plants in V5/V8/R1 no disease was found. On the fourth evaluation of foliar disease at R4, corn plants in CON had an average of 6% of leaf area infected with Northern Leaf Blight, 1% of leaf area detected with common rust; corn plants in V5 had an average of 3.5% of leaf area infected with Northern Leaf Blight, an average of 1% of leaf area infected with common rust V5, and an average of 1.3% of leaf area infected with Gray Leaf Spot. No signs of foliar disease were found in V5/V8 and V5/V8/R1 at the fourth evaluation.

All treatment averages and contrast for the digestibility experiment seen in Table B.1. No difference was observed for the fraction of soluble corn silage in CON when compared to corn silage treated with foliar fungicide application ($P = 0.47$). The undegradable portion did not differ for corn silage in CON compared to corn silage in treatments with foliar fungicide application ($P = 0.58$). Furthermore, no difference was observed among treatments for the rate at which corn silage is digested ($P = 0.47$). A diagram for the DM degradability for corn silage in all four treatments is shown in Figure B.1.

DISCUSSION

The purpose of this experiment was to evaluate the effect of foliar fungicide applied at various times during the growth and development of corn, then ensiled as corn silage, on the ruminal DM degradability; measured using the *in situ* technique.

During the 2014 corn growing season, very little disease was seen in the field. The maximum amount of disease only accounted for 6% of the leaf area in CON. In a meta-analysis, when disease was greater than 5% of the total leaf area, corn yields suffered more compared to when disease was less than 5% of the total leaf area (Paul et al., 2011). Furthermore, applications of fungicide on corn with a higher disease severity had an increase of 114 to 400 kg/ha mean yield compared with growing seasons where disease was less than 5% of the leaf area (Paul et al., 2011). Therefore, if the weather conditions had been favorable for fungal growth, the results for the digestibility of corn silage with foliar fungicide application compared to untreated corn silage may have been different. When the plant is under fungal pressure, the plant begins the lignification process of the cell wall, as a barrier to preventing fungal enzymes from metabolizing plant cell nutrients. As evidence to this, Wang et al. (2010) evaluated the true digestibility of corn silage from corn inoculated with Northern Leaf Blight compared to uninfected corn silage, and reported DM and NDF digestibility decreased for sheep fed diseased corn silage. The amount of disease on the plant material is a major difference between our study and Wang et al. (2010); as the disease in Wang et al. (2010) was purposely inoculated on the plant, when in the current study it was left to environmental conditions. Furthermore, Bal et al. (2000) evaluated two hybrids of corn using *in situ* technique; one corn hybrid with a high concentration of NDF and the other with a low concentration of NDF. Corn silage from corn with a low NDF concentration had a greater DM ruminal disappearance when compared with corn silage with the high NDF concentration. In the future, inoculating plants with a fungus in the field may allow us to see a greater difference in the DM digestibility.

No differences in the soluble fraction, digestible fraction, or undegradable fraction were observed for corn silage with foliar fungicide treatment when compared to control (Table B.1). The soluble portion in the current study is a smaller proportion of the feedstuff when compared to the results of others. The fraction of soluble feed of corn silage in Haerr (2015) ranged from 0.35 to 0.23. Furthermore, Philippeau and Michalet-Doreau (1998) reported the soluble portion of corn silage from dent corn and flint corn to be 37% and 31%, respectively. Variation between studies may have been due to temporal and bag conditions within the rumen of the cow (Vanzant et al., 1998). In the future, it may be best if small bags and large bags containing ground and unground corn silage (not included due to many of the bags ripping) are not included in the rumen at the same time to allow for complete access to fermentation.

When modeling *in situ* experiments, a lag time in digestion can help to explain an initial gap in time where the microbes are adapting to the substrate (Van Soest, 1994). A lag time equation was initially used in the current study, but estimates returned from NLREG did not differ from zero. Peyrat et al. (2014) did not observe a lag time in the DM degradation of corn silage measured *in situ*, but did observe a lag time in the NDF degradation of corn silage measured *in situ.* Haerr (2015) showed a lag time ranging from 19 min to 3.9 h in the DM digestibility of corn silage measured *in situ.* Again, variation in techniques and animal rumen conditions could be a reason why discrepancy exists among studies.

In future *in situ* studies, using undried corn silage instead of dried corn silage in the nylon bags may give a better estimation of the fermentation rates (Cherney and Cherney, 2003). Furthermore, forage comprised 57% of dietary DM. Including a greater proportion of forage in the diet of the cows on *in situ* digestibility study could allow for greater cellulose activity in the rumen and therefore, a more concentrated microbial population compared to diets with lower forage concentrations. Vanzant et al. (1998) recommends feeding forage at 60 to 70% of diet to cows included in an *in situ* experiment.

CONCLUSION

Significant foliar disease was not observed for corn in any treatment. Foliar fungicide application on corn ensiled, as corn silage had no effect on the DM digestibility of the soluble fraction, the digestible fraction, or the undegradable fraction when compared to corn silage with no fungicide application, measured *in situ.*

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TABLES AND FIGURES

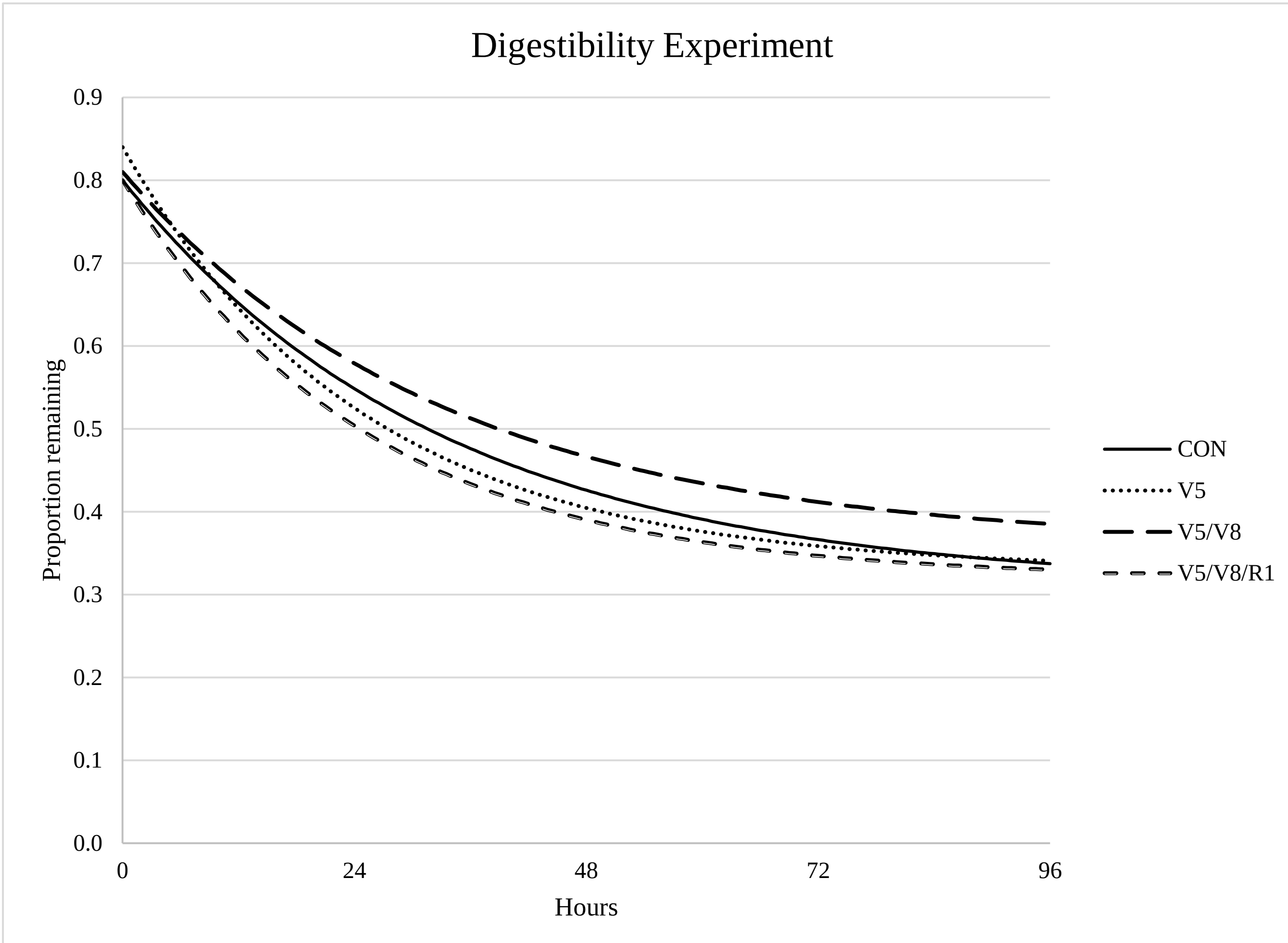
Table B.1. Least squares means and associated standard errors for soluble feed portion, digestible feed portion, undegradable feed portion, and fractional rate of digestion (K_d) for corn silages treated with no foliar fungicide (**CON**), one application of foliar fungicide (**V5**), two applications of foliar fungicide (**V5/V8**), or three applications of foliar fungicide (**V5/V8/R1**).

	Treatments[1]					**P-Value**		
						Contrasts[2]		
	CON	**V5**	**V5/V8**	**V5/V8/R1**	**SEM**	**CON vs. TRT**	**V5 vs. V5/V8**	**V5/V8 vs. V5/V8/R1**
DM								
Soluble	0.20	0.15	0.18	0.21	0.02	0.47	0.19	0.24
Digestible	0.49	0.51	0.45	0.48	0.03	0.79	0.14	0.55
Undegrabable	0.31	0.33	0.36	0.32	0.03	0.58	0.57	0.32
K_d, h^{-1}	0.03	0.04	0.03	0.04	0.009	0.47	0.78	0.64

[1] Treatment = Dietary treatments were CON (with no application of fungicide), V5 (with 1 application of fungicide at V5), V5/V8 (with two applications of fungicide at V5 and V8), and V5/V8/R1 (with three applications of fungicide at V5, V8, and R1).

[2] Contrasts were Contrasts were CON vs. TRT = no fungicide application (CON) with that of the average of the three treatments with fungicide application; V5 vs. V5/V8= fungicide application at V5 compared with adding fungicide at both V5 and V8; V5/V8 vs. V5/V8/R1= fungicide application at V5 and V8 compared with the to the treatment where fungicide was applied at V5, V8, and R1.

Figure B.1. *In situ* digestion kinetics for the proportion of DM remaining after ruminal incubation of corn silage in CON, V5 (with one application of foliar fungicide), V5/V8 (with two applications of foliar fungicide), and V5/V8/R1 (with three applications of foliar fungicide).



Appendix C

Corn root radius and weight with foliar fungicide

Kalebich, C., and F. Cardoso

INTRODUCTION

Applications of fungicide on corn may affect root growth and nutrient uptake by the plant from the soil. Further experiments should be conducted to scientifically evaluate relationships. Results from roots collected during the summer of 2015 cannot be published as collection did not occur in a replicated manner. During the summer of 2015, I had not yet had a statistics class where the importance of replication was discussed and miscommunication led to this failure. I understand the mistake made; it was a lesson learned.

MATERIALS AND METHODS

During the summer of 2015, corn roots identified and removed from the field in an identical manner. Once plants were located and stalks removed for experiments in Chapter 3, a 25.4-cm square was dug around the residue and root system (Figure C.1). Roots were gently rocked back and forth in the ground and pulled straight up, careful not to break the roots. Once roots were removed, roots were brought back to the laboratory at the dairy to be wash soil off and analyze. The radius of a root system was starting at the stalk to the longest root within the same (Figure C.2 and Figure C.3). Weight of clean root samples was also collected.

RESULTS

Preliminary results from root collection at both R1 and R3 are seen in Table C.1. Preliminary data for corn roots shows fungicide applications on corn have smaller root weights (204.1, 260.8, and 272.7 g for V5, V5+R1, and R1, respectively) and smaller root radii (24.6, 29.6, and 27.1 cm for V5, V5+R1, and R1, respectively) than untreated corn (335.9 g for weight, and 39.0 cm for radius) at the R1 collection. Results from the R3 collection also report applications of fungicide on corn have smaller root weights (133.2, 113.4, 275.0 g for V5, V5+R1, and R1, respectively) and smaller root radii (22.9, 43.2, 31.3 cm for V5, V5+R1, and R1, respectively) than untreated corn (330.3 g for weight, and 48.3 cm for radius).

CONCLUSION

Corn roots with foliar fungicide application may have been smaller in radius and weighed less.

TABLES AND FIGURES

Figure C.1. Collection of corn roots in the field.

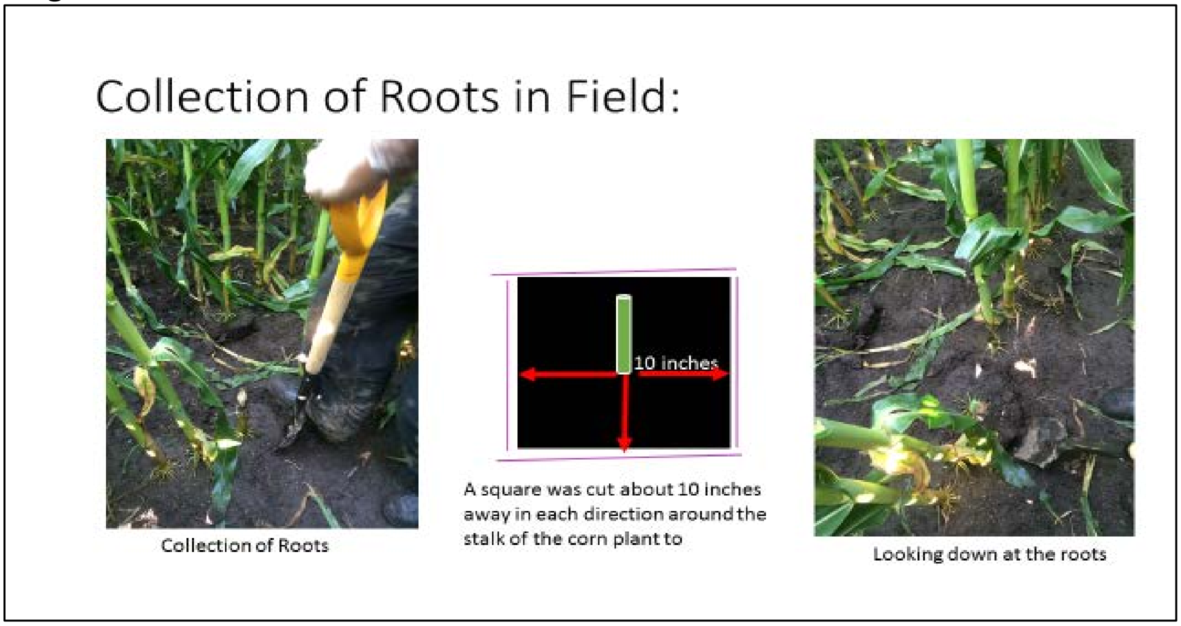


Figure C.2. Radius of corn root system from bottom.

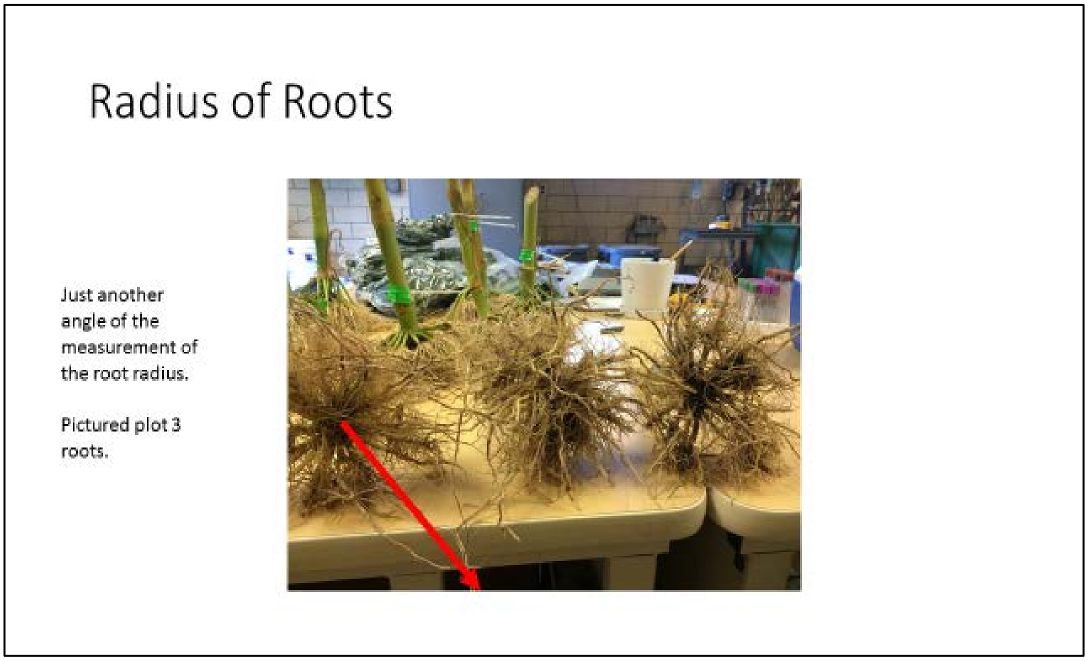


Figure C.3. Radius of corn root system from the top.

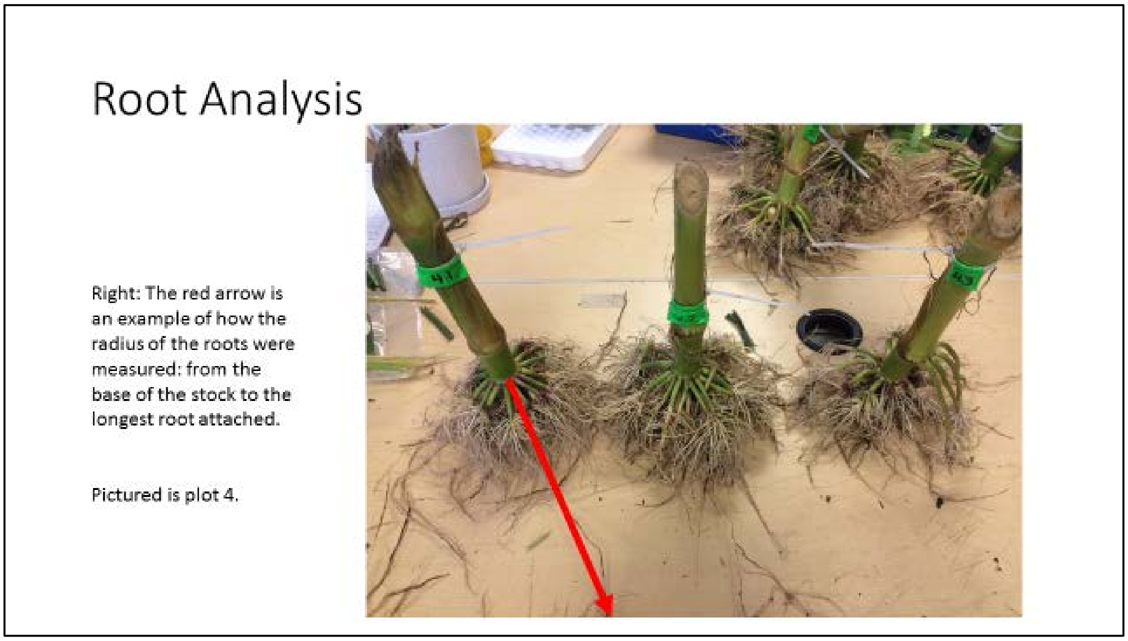


Table C.1. Averages and standard deviations for roots in (CON), one application of fungicide at V5 (V5), two applications of fungicide at V5 and R1 (V5 + R1), one application of fungicide at R1 (R1).

	Treatment				
	CON	**V5**	**V5+R1**	**R1**	**SD**
R1 Stage					
Weight[1]	335.94	204.12	260.82	272.72	74.28
Radius[2]	38.95	24.55	29.63	27.09	3.33
R3 Stage					
Weight	330.27	133.24	113.40	274.99	145.72
Radius	48.26	22.86	43.18	31.33	20.79

[1]Weight measured in grams
[2]Radius measured in centimeters